TECHNOLOGIES AND NOVEL METHODOLOGIES FOR THE ASSESSMENT OF NUTRITIONAL INTERVENTIONS

FIONA L DODD

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Technologies and Novel Methodologies for the Assessment of Nutritional Interventions

Fiona L Dodd

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Abstract

The aim of this thesis was to identify novel techniques in the assessment of nutritional intervention effects upon cognition. The impact of combining different cognitive and physiological assessments of nutritional interventions was explored in order to establish whether it could provide a more detailed picture of any effects, as well as the mechanisms by which they may occur.

This thesis initially used two different technologies, electroencephalography (EEG) and near infrared spectroscopy (NIRS) to assess the cerebro-electrical and haemodynamic impact of cognitive task performance following *Ginkgo biloba and a Ginkgo biloba/Panax ginseng* combination, in healthy young adults. Following on from this, the effects of two different doses of *Ginkgo biloba* were investigated on cerebral blood flow and oxygenation parameters during the repeated administration of cognitively demanding tasks. The synergistic effect of two interventions believed to possess disparate effects on cerebral blood flow; caffeine and L-theanine, were then assessed during the performance of a range of cognitive tasks. To evaluate the peripheral as well as the central impact of task performance, a further assessment of (two doses) caffeine was conducted whilst cerebral blood flow and oxygenation parameters were monitored alongside an assessment of metabolism via indirect calorimetry (ICa). In an extension of the methodology, an exercise element was incorporated into the protocol and beetroot juice was administered whilst cerebral blood flow and haemodynamics were monitored during task performance, before, during and after cycling at different exercise intensities.

The results of this thesis have identified that the methodologies adopted are capable of detecting changes in cerebral oxygenation as a result of, nutritional challenge; differing doses of the same intervention; the synergistic effect of two different interventions, and during incremental exercise whilst performing cognitive tasks. The concomitant measurement of NIRS and ICa were also shown to be effective in simultaneously determining the somatic and cognitive demands of a task. These findings demonstrate the positive contribution to research of combining technologies and methodologies in the assessment of nutritional interventions and provide valuable information in respect of their use in cognitive research.

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List of Tables and Figures

Publications and Conference Proceedings

Data within this thesis has formed the basis of the following peer reviewed publications:

Dodd FL, Kennedy DO, Riby LM, Haskell-Ramsay CF (2015) A double-blind, placebo-controlled study evaluating the effects of caffeine and L-theanine both alone and in combination on cerebral blood flow, cognition and mood. Psychopharmacology 232(14): 2563-76

Thompson KG, Turner L, Prichard J, Dodd F, Kennedy DO, Haskell C, Blackwell JR, Jones AM (2014) Influence of dietary nitrate supplementation on physiological and cognitive responses to incremental cycle exercise. Respir Physiol Neurobiol. 193:11-20.

Conference Proceedings

An evaluation of the cerebral blood flow, cognitive and mood effects of caffeine and L-theanine both alone and in combination.

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Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others. For Chapter 2, funding was received from Merz Consumer Care GmbH, Frankfurt. Chapter 6 was conducted in collaboration with the Department of Sport, Exercise and Rehabilitation; however, all elements presented within this thesis are the authors own work.

The research presented in this thesis has received ethical approval from the Faculty of Health and Life Sciences Ethics Committee at Northumbria University.

I declare that the Word Count of this Thesis is 58,462 words.

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Chapter 1. Introduction

1.1 General Introduction

The influence of diet upon physical and mental health has been of interest for a number of years. However, with an ageing population, there is increased interest in the use of nutraceuticals, functional foods and nutritional interventions that can modulate cognition or physiological parameters in a positive or indeed, negative way. There are many methodologies that can be adopted and technologies that can be employed in order to measure behavioural and or physiological outcomes. Yet, either because of practical or technological limitations, their implementation in combination with nutritional interventions of interest to cognition has, in some cases, been overlooked. The benefit of combining different methods of cognitive and physiological assessment is that a more thorough and detailed picture of the effects and the mechanisms by which the effects occur can be obtained. A workshop by the Nutrition and Mental Performance Task Force of the European branch of the International Life Sciences Institute took place in 2009 with the aim of pinpointing gaps in our knowledge in relation to cognitive performance assessment and identifying nutritional and methodological opportunities for improving brain function and cognitive performance. A number of recommendations focussed on improving current approaches were made, including, the use of biomarkers and risk factor measures for cognitive impairment to supplement clinical or cognitive measures; a greater focus on the prevention as opposed to the treatment of cognitive decline (i.e. targeting younger people); focussing on particular cognitive tests that are relevant to the areas of brain function at which the intervention is aimed; more widespread use of computerised cognitive tests in order to standardise administration and provide parallel versions of tasks; the delivery of tasks in multiple languages and the inclusion of brain imaging techniques as biomarkers in order to supplement cognitive methods.

Proposals such as this highlight the importance of reviewing our methodological approach to nutrition and cognition research. In cognitive assessments of nutritional interventions, creating opportunities to integrate methodologies and technologies enables an assessment of the ‘bigger picture’. By consequence, we are then able to determine at

more than one level, the intervention in questions' ability (or indeed absence of ability) to convey cognitive and or physiological benefits, both in the short and the long-term.

1.2 Methods of assessing nutritional interventions of interest to cognition in healthy young populations

1.2.1 Cognitive Tasks

With a vast number of nutritional interventions having the ability to modulate cognition, the approach used to elicit and measure these effects is wide ranging. They may include simple assessments looking at the effects upon an individual outcome or the use of complex paradigms incorporating different technologies where any number of behavioural and/or physiological outcomes are assessed. From the simplest study to the most complex however, when there is an outcome determining behavioural performance, this is typically measured by the use of cognitive task(s), with or without an assessment of subjective mood.

1.2.1.1 Considerations in the use of cognitive tasks when assessing the effects of nutritional interventions

Frequently, within nutritional intervention studies where the main focus is the cognition enhancing properties of the intervention, the primary outcome measures centre around behavioural performance on one or more cognitive tasks. The decision to include a task is usually taken based upon the tasks ability to assess a specific area of cognition (for example, attention or memory). It also commonly reflects prior knowledge of the interventions ability to modulate this area. When selecting appropriate tasks for use within a nutritional intervention study, the tasks should have previously demonstrated their sensitivity to the nutritional intervention in question, in addition to their validity and reliability. Task sensitivity is an important consideration when assessing the effects of any nutritional intervention since the observed effects may be very subtle. Good practice therefore dictates that only those tasks that have reliably, and (preferably) more than once demonstrated their ability to detect a change in cognitive function, should be included (Wesnes, 2010). Due to a strong association with sensitivity, measures also need to

demonstrate their reliability for retesting. They should therefore exhibit little variation after repeated assessment, since greater variability in performance can adversely affect the detection of a significant effect as a result of nutritional challenge (Schmitt, Benton, & Kallus, 2005). The validity of a task - that a task is measuring what it purports to measure (i.e. that a task of attention is indeed measuring attention) is also a minimum requirement when planning what measures should be included within any interventional study. Another important consideration is mode of presentation, since in some instances, paper and pencil as well as automated versions may be available. The advantages of computerised versions are that the presentation of the tasks can be standardised and responses can be captured in a detailed and accurate manner (Schmitt et al., 2005), allowing less room for human error during scoring. Within nutritional cognitive research, the speed at which participants respond to a stimulus can be equally as interesting as their level of accuracy. Computerised testing facilitates this by providing the capability to record reaction times alongside other measures and identify reaction times more precisely. It also enables the inclusion of tasks such as choice or simple reaction time (Wesnes, 2010). Computerised systems with the capability of delivering a battery of tasks also minimise researcher input/interruption thereby preventing or at least limiting any researcher bias. Overall, this leads to a smoother delivery and efficient transition between tasks. All of these considerations are important when planning a nutritional intervention study with a cognitive element, as they ensure good practice and increase the likelihood of observing an effect of treatment.

1.2.2 Manipulating cognitive demand/fatigue

Age-related cognitive decline is a feature of ageing that is well documented (Craik & Salthouse, 2008; Salthouse, 1991; Salthouse & Babcock, 1991), with some aspects of decline appearing as early as between 20 and 30 years of age (Salthouse, 2009). By contrast, healthy young adults who are at the peak of their cognitive abilities and have not yet entered a phase of cognitive decline are more susceptible to ceiling effects during assessments of behavioural performance. This can be problematic when attempting to

assess the cognition-modulating properties of an intervention as any effects could be masked. One approach when testing healthy young participants, who by their nature should be at the peak of their abilities, is to introduce a level of cognitive demand or fatigue to the protocol. Assessing the effects on performance of an intervention in this way can highlight its capacity to recover function, in addition to its ability to prevent continued decline in performance. Introducing a degree of cognitive demand through the use of complex tasks, tasks of increasing difficulty or the repeated performance of tasks is one means of manipulating demand with a view to increasing fatigue. It has previously been identified that there exists a relationship between the delivery and use of glucose and oxygen, in that both can facilitate cognitive performance (Kennedy & Scholey, 2000; Moss & Scholey, 1996; Moss, Scholey, & Wesnes, 1998; Scholey, Harper, & Kennedy, 2001; Scholey, Moss, Neave, & Wesnes, 1999). This effect is found to be more apparent when the level of cognitive demand is high (Kennedy & Scholey, 2000; Scholey et al., 2001). With the aim of manipulating the level of effort required during behavioural performance, the same research group as referred to above, introduced a group of tasks called the 'Cognitive Demand Battery' (CDB). This battery consisted of 2 minutes each of the performance of serial 3s and serial 7s subtractions, followed by 5 minutes of the rapid visual information processing (RVIP) task followed by a subjective mental fatigue scale. This set of tasks was then repeated six times over the period of an hour. The purpose of this battery was to increase mental fatigue, producing a state in which cognitive performance in healthy young participants was likely to decline. Using this method, significant improvements in cognitive performance have been demonstrated in healthy young adults following the administration of glucose*, Panax* ginseng and cocoa flavanols (Kennedy & Scholey, 2004; Reay, Kennedy, & Scholey, 2005, 2006; Scholey et al., 2009).

1.2.2.1 Exercise, cognition and fatigue

A further approach is to manipulate demands on the body through the use of physical exercise. Including an exercise element in the protocol during the performance of cognitive tasks introduces an additional level of fatigue. Studies that have adopted this methodology have aimed to determine the acute and longitudinal effects of physical

exercise on cognitive performance. The importance of this approach has been validated by coaches and athletes alike, since attentional and strategic choices that have to be made in a short space of time can be key to successful performances in sport (Brisswalter, Collardeau, & Rene, 2002). Research within this field has incorporated an assessment of cognition either prior to the onset of exercise, whilst participants are actively exercising, during the post-exercise recovery period, or via a combination of these three paradigms. Due to potential practical limitations such as instrument/equipment set-up, it could be suggested that the more challenging approach is measuring cognitive function whilst participants are actively exercising. However, cognitive task performance has been successfully measured during a range of exercise methods: simple reaction time during cycling at 20-80 % of maximal aerobic power (Brisswalter, Arcelin, Audiffren, & Delignieres, 1997) and whilst running on a treadmill (Bender & McGlynn, 1976; Collardeau & Brisswalter, 2001); performing a choice reaction time task during moderate sub-maximal cycling (Arcelin, Delignieres, & Brisswalter, 1998; Davranche & Audiffren, 2004; Davranche, Audiffren, & Denjean, 2006) and during prolonged cycling (Chmura, Krysztofiak, Ziemba, Nazar, & Kaciuba-Uscilko, 1997); and completing a speed of visual search task whilst cycling at 70 % and 100 % maximal power (McMorris & Graydon, 1997).

The influence of nutrition and nutritional supplementation upon exercise is an area of research that has received attention for many years, with interventions such as caffeine and carbohydrates known to positively facilitate aspects of exercise performance (Glaister et al., 2008; Karelis, Smith, Passe, & Peronnet, 2010). However, comparatively few studies have monitored cognition whilst participants are actively exercising, in the presence of a nutritional intervention known to improve either cognitive or exercise performance. Those that have, have used attentional and reaction time tasks during prolonged exercise, such as cycling, running or aerobic activity, in the presence of caffeine or carbohydrates (Collardeau, Brisswalter, Vercruyssen, Audiffren, & Goubault, 2001; Hogervorst et al., 2008; Lieberman, Falco, & Slade, 2002) and have demonstrated positive results on cognition in some cases (Hogervorst et al., 2008; Lieberman et al., 2002).

1.2.2.1.1 Indirect calorimetry (ICa)

Indirect calorimetry (ICa) is a non-invasive and portable method of determining estimates of energy expenditure during exercise and resting states from inspired and expired air. The method is termed indirect since energy expenditure readings are obtained as a result of formulas derived from measures of oxygen consumption and carbon dioxide production as opposed to a direct measure of heat loss (Reaburn, Reed, Dascombe, Jones, & Weyers, 2011). The use of this method is popular within exercise and nutrition research protocols (Acheson, Zahorskamarkiewicz, Pittet, Anantharaman, & Jequier, 1980; Astrup et al., 1990; Hodgson, Randell, & Jeukendrup, 2013; Hollands, Arch, & Cawthorne, 1981; Saffle et al., 1985) as well as within clinical settings (Garcia-Peris et al., 2005; Miller, Daniels, Bannerman, & Crotty, 2005; Reeves, Battistutta, Capra, Bauer, & Davies, 2006), where it remains the gold standard for measuring energy expenditure (Haugen, Chan, & Li, 2007).

1.3 Technologies for assessing interventions of interest to cognition

During cognitive testing, there are a range of neurophysiological methods and techniques that may also be adopted. These techniques can be used either alone or in conjunction with one another, in order to provide a fuller picture of a supplements' ability to modulate cognition.

1.3.1 Measuring cerebral activation and blood flow

Cerebral blood flow (CBF) controls the delivery of metabolic substrates (glucose and oxygen) to the brain (Scholey, 2001). At the simplest level, increases in CBF, oxygen delivery and consumption occur when the metabolic demand of neurons change as electrical signals are passed between cells due to neuronal activation (Lloyd-Fox, Blasi, & Elwell, 2009). Fox et al, (1988) were the first to identify that the resulting increase in blood flow exceeds the rate at which oxygen is utilised and as a result leads to an increase in oxygenated haemoglobin (oxy-Hb) in activated areas. In terms of the haemodynamic response during functional activation, this is generally demonstrated by an increase in

oxygenated haemoglobin and a corresponding decrease in deoxygenated haemoglobin (deoxy-Hb) in the area of activation (Obrig et al., 2000).

1.3.1.1 Ageing and cerebral blood flow

Cerebral blood flow is a measurement that is susceptible to change dependent upon the population being assessed. Age differences have been implicated as having an influence on CBF. Cerebral blood flow is thought to reduce with age, and a number of studies, using different methodologies, including positron emission tomography (PET) (Leenders et al., 1990; Marchal et al., 1992; Martin, Friston, Colebatch, & Frackowiak, 1991), single photon emission tomography (SPET) (Larsson et al., 2001) and 133Xenon inhalation method (Melamed, Lavy, Bentin, Cooper, & Rinot, 1980) have demonstrated this. However, there is also research to suggest that CBF remains stable, even with advancing age (Itoh et al., 1990; Yamaguchi et al., 1986). As a consequence, within the present thesis in order to remove age as a confounding factor, only young, healthy populations were assessed.

1.3.2 Functional magnetic resonance imaging (fMRI)

Functional Magnetic Resonance Imaging (fMRI) uses magnetic resonance imaging technology to examine changes in blood flow in a specific brain region as a direct result of stimulation, for example during performance of a cognitive task. The technique of fMRI relies on the assumption that changes in neural activity are closely related to changes in blood flow. Therefore, as neural activity increases there is a corresponding increase in blood flow in that area of the brain. Blood Oxygen-Level Dependent (BOLD) contrast fMRI was developed by Ogawa et al., (1993) and uses the magnetic property differences of deoxy-Hb and oxy-Hb to identify changing signal variations as a result of activation. It is currently considered the gold standard for measuring functional brain activation, due predominately to the high level of spatial resolution fMRI provides (Bunce, Izzetoglu, Izzetoglu, Onaral, & Pourrezaei, 2006). However, there are limitations to this method including low temporal resolution, as data collected is limited to the order of seconds as opposed to milliseconds. It is also very restrictive and participants are required to lie very still to minimise movement and prevent contamination of the data. As a consequence, the

tasks and protocols that can be utilised in conjunction with fMRI are limited. fMRI has been used frequently in cognition research in order to identify the neural correlates of performing a task (Buckner et al., 1996; Dove, Pollmann, Schubert, Wiggins, & Yves von Cramon, 2000; Milham et al., 2002; Rissman, Gazzaley, & D'Esposito, 2004) and in the absence as well as in the presence of nutritional interventions (Chen & Parrish, 2009b; Francis, Head, Morris, & Macdonald, 2006; Laurienti et al., 2002).

1.3.3 Electroencephalography (EEG)

Electroencephalography (EEG) is a non-invasive measurement of electrical activity in the scalp as a result of the activation of neurons. This activation is captured as electric potential differences between numerous pairs of electrodes placed at specific points on the head that correspond to different regions of the brain. The electrical activity shows a pattern of oscillations, displayed as waves of electrical potential at a variety of frequencies and amplitudes. These characteristics are well established and can change depending on the situation and the brain location in which the recordings take place, for example, frontal or occipital, awake versus sleeping, eyes open versus eyes closed etc. (Lieberman, Kanarek, & Prasad, 2005). The technique of EEG can also record electrical activity that is associated with a specific external stimulus (sensory, cognitive, motor–related etc.) and this type of response is known as an event-related potential (ERP). It is recorded in the same way as spontaneous activity (via use of electrodes placed on the scalp); however, it is measured at the precise time the external stimulus takes place and it displays a characteristic waveform (Luck, 2014). Benefits of this technique include a high temporal resolution including the monitoring of changes in electrical activity within milliseconds. It is silent and is not as susceptible to contamination from artefacts as a result of movements. In comparison to some imaging techniques such as fMRI, it is also low cost. However, it does have disadvantages, including low spatial resolution - making it difficult to clearly identify specific areas of the brain that are activated. The set-up of EEG can also be time-consuming, depending upon the number of electrodes used in a study, as they may have to be connected individually. EEG is most commonly used in the diagnosis of medical

conditions such as epilepsy and coma (Salinsky, Kanter, & Dasheiff, 1987; Smith, 2005; Young, 2000). However, EEG is also used in cognition research as well as in the presence of nutritional interventions (Brown & Riby, 2013; Kennedy et al., 2003; Riby et al., 2008; Smith, Riby, Sunram-Lea, Van Eekelen, & Foster, 2009).

1.3.4 Near infrared spectroscopy (NIRS)

Although, as previously discussed, fMRI is considered the current gold standard for measuring brain activation, in certain situations, or indeed, within some populations, the requirements of fMRI monitoring cannot be met. In this instance, NIRS is emerging as an alternative imaging technique, due to its practical application and high temporal resolution. An alternative to brain imaging techniques such as fMRI and positron emission tomography (PET); methods which have been described as expensive, physically constraining, and in the case of PET, may expose participants to potentially harmful materials (Izzetoglu, Yurtsever, Bozkurt, & Bunce, 2003). NIRS is a safe, portable, non-invasive, negligibly intrusive, and (comparatively) inexpensive method of imaging that does not require participants to be confined to restricted positions. It also provides a more thorough assessment of the haemodynamic response as (unlike fMRI) it gives measurements of both oxy-Hb and deoxy-Hb (Lloyd-Fox et al., 2009). The use of NIRS for a variety of applications has become increasingly popular since its inception over 20 years ago, and consequently the number of NIRS publications has doubled every 3.5 years since (Boas, Elwell, Ferrari, & Taga, 2014). This is perhaps due in part, to its applicability within certain situations or populations where other imaging techniques (such as fMRI, PET etc) would not be suitable, for example in young populations such as neonates, babies and toddlers (Gervain et al., 2011). NIRS has also been successfully adopted as a monitoring tool during (non-cardiac) surgery to monitor cerebral saturation (Moerman & De Hert, 2015) and during exercise, at a range of intensities (Ide, Horn, & Secher, 1999; Miyai et al., 2001; Subudhi, Dimmen, & Roach, 2007; Suzuki et al., 2004) including maximal (Subudhi et al., 2007; Subudhi, Miramon, Granger, & Roach, 2009; Thomas & Stephane, 2008). In the context of the current thesis, NIRS is also suitable for

use during cognitively demanding situations that require complete concentration. Since, in contrast to fMRI, NIRS is silent, thereby limiting the distraction to participants during cognitive tasks and enabling the presentation of stimuli both visual and auditory. In addition, NIRS can be used repeatedly with the same participants, making it useful for repeated measures designs and suitable for the study of cognition related haemodynamic changes (Izzetoglu et al., 2003). It also allows the assessment of interpersonal activation patterns through the monitoring of simultaneous haemodynamic responses as a result of two-person experimental paradigms. For example, when two participants perform a dual task, that may have an advantage over the single performance of that task (Dommer, Jaeger, Scholkmann, Wolf, & Holper, 2012; Holper, Scholkmann, & Wolf, 2012).

1.3.4.1 Principles of NIRS

Near infrared spectroscopy measures functional activation through monitoring changes in the haemodynamic properties of the brain (Huppert, Hoge, Diamond, Franceschini, & Boas, 2006). Three distinct types of near infrared implementation have been developed: time-resolved systems, frequency domain systems and continuous wave spectroscopy systems, each of which has its own strengths and limitations (Bunce et al., 2006). A review of the different methods is beyond the scope of this thesis; however, see Delpy & Cope (1997) for further information. The method that will be discussed in the following chapter and throughout this thesis is continuous wave spectroscopy. This method of spectroscopy works via the application of continuous or slow pulsed light to tissue (Bunce et al., 2006) and enables the observation of changes in regional cerebral blood flow as it happens through the measurement of concentration changes in oxy-Hb, deoxy-Hb and total-Hb (calculated from the values of oxy-Hb and deoxy-Hb) (Hoshi, 2007). In the near-infrared range of 700-1000 nm, biological tissue is largely transparent to light. This non-invasive imaging technique uses two wavelengths of light that are differentially absorbed by oxy-Hb and deoxy-Hb haemoglobin (wavelengths of ~830 and 750 nm and are generally adopted in NIRS research (Boas, Dale, & Franceschini, 2004)). They are introduced through the skull via a laser emitter and measured following transit through the upper surface of the cortex by an optode (termed the receiver) placed at a pre-set

distance from the light source (Bunce et al., 2006). As light travels through the tissue, some is absorbed and some is scattered, the light that remains is collected by the receiver (which is normally placed 2-7 cm away from the emitter), once it has passed through the tissue beneath the optodes (Villringer & Chance, 1997). See figure 1.1 for an example of the 2-channel NIRS set-up over the pre-frontal cortex, used in the current thesis. The number of channels used within a study varies depending upon the brain area under investigation and paradigm being assessed, with systems having been developed that can measure up to 128 channels (Lloyd-Fox et al., 2009). The concentration changes of oxy-Hb and deoxy-Hb can be calculated from the amount of light absorption at their specific wavelengths via the modified Beer-Lambert law (Fallgatter & Strik, 1998). The Beer-Lambert law is an empirical description of optical attenuation in a highly scattering medium (Bunce et al., 2006). At a distance of 4 cm between emitter and receiver optodes, the NIRS signal becomes sensitive to haemodynamic changes within the top 2-3 mm of the cortex extending laterally 1 cm to either side (Chance et al., 1998).

Figure 1.1 Example of 2-channel NIRS device and configuration of optodes over pre-frontal cortex as used in the current thesis.

1.3.4.2 Limitations of NIRS

NIRS, however, does have its limitations. One of the main criticisms of NIRS and a significant challenge facing functional near infrared spectroscopy (fNIRS) research is its low spatial resolution. As a result, defining the precise area of brain under investigation is difficult and fNIRS studies have had to rely on the international 10-20 system to roughly deduce the area being measured (Hoshi, 2007). The 10-20 system uses anatomical markers such as nasion, inion and preauricular points in the placement of electrodes. It is

based on the association between the position of an electrode and the underlying area of the cerebral cortex. The '10' '20' in the title relates to the fact that the electrodes are spaced to the adjacent electrode at distances of either 10 or 20 % of the total front-back, right-left distance of the skull (Klem, Lüders, Jasper, & Elger, 1999). NIRS also has low depth penetration thereby limiting assessment of cerebral activation to the cortical grey matter (Villringer & Chance, 1997). This factor has bearing upon the distance between transmitter and receiver optodes, since to reach the cortex the distance between the source and the detector optodes must be (at a minimum), double that of the distance between the skin surface and the surface of the cortex (Lloyd-Fox et al., 2009). As a consequence, distances between transmitter and receiver optodes are usually fixed at a set distance within individual fNIRS studies. However, there is large inter-subject variability in the dimensions of subjects' foreheads. This, combined with differences in brain size mean that identifying the exact area under investigation where haemodynamic changes are taking place can be difficult (Izzetoglu, Bunce, Onaral, Pourrezaei, & Chance, 2004). Motion artefacts and the method by which they are handled (if indeed at all), is another of the challenges faced in fNIRS research. Motion artefacts are defined as "abrupt changes in signals occurring in several channels simultaneously which are distinctive from the slow and smooth haemodynamic response that is usually seen" (Lloyd-Fox et al., 2009). Such artefacts are caused by displacement (however slight) of the optodes, usually as a result of head movements, body movements, involuntary actions (yawning, sneezing etc.) or touching of the optodes when the device is in use. As a result, external light may be detected by the receiver, or light may pass from the transmitter to the detector without passing through the tissue (Izzetoglu et al., 2004). Head movements can also result in changes in CBF and alterations in oxygen levels that can mimic haemodynamic changes as a result of brain activation and these can be almost indistinguishable from the normal response (Izzetoglu, Devaraj, Bunce, & Onaral, 2005). Unfortunately there is currently no universal approach for dealing with motion artefacts in fNIRS research, as with other imaging techniques (Robertson, Douglas, & Meintjes, 2010). There are, however, a number of methods proposed to minimise the impact of such

artefacts. One of the simplest is to visually identify where the artefact occurs (via a time stamp, block of data or participant) and remove this data from the analysis (Nakano, Watanabe, Homae, & Taga, 2009). Alternative techniques include wavelet-based filtering (Molavi & Dumont, 2012; Robertson et al., 2010) or the use of algorithms (Izzetoglu et al., 2005). Other approaches have focussed on procedures that can be adopted in order to reduce the occurrence of artefacts. Methods include the use of a light absorbing band (Fallgatter & Strik, 1998) and adhesive fixation discs that secure the headband to the skin. However, criticisms remain that even though improved development of the headband/connection of the optodes to the skin will reduce the occurrence of artefacts, it is unlikely to fully solve the issue (Lloyd-Fox et al., 2009).

1.3.4.3 Functional near infrared spectroscopy (fNIRS)

Functional optical imaging is the assessment of physiological changes associated with brain activity by optical methods (Villringer & Chance, 1997). The ability of NIRS to measure a hemodynamic change during motor, visual and auditory stimulation (Cutini, Scatturin, Basso Moro, & Zorzi, 2014; Herrmann, Ehlis, Wagener, Jacob, & Fallgatter, 2005; Kotilahti et al., 2005; Obrig et al., 1996; Sakatani, Chen, Lichty, Zuo, & Wang, 1999; Taga, Asakawa, Hirasawa, & Konishi, 2003), in addition to more subtle processes such as deception (Izzetoglu et al., 2002; Tian, Sharma, Kozel, & Liu, 2009) has been validated. Of specific relevance here, however, is the capacity of NIRS to identify cerebral activation as a result of cognitively demanding tasks. Izzetoglu et al. (2003) used NIRS across the forehead in a 4 x 10, 16 channel design to monitor cerebral haemodynamics in the frontal region of the brain during tasks which assessed attention and working memory (target categorisation and N-back) (see figure 1.2 for example of a multi-channel NIRS configuration). Cognitively intact males aged 18-35 years completed either a target categorisation task (n=7) or the n-back task (n=4). Oxy-Hb was seen to increase with increasing task difficulty during the n-back task (0 back – 2 back) with a reduction in oxy-Hb during 3-back. It was concluded that, in the case of the most difficult version of the task, the demands required exceeded the subject's ability to keep up leading to a decrease in oxygenation. There was no report of measurements of deoxy-Hb or total-Hb.

A later study by the same research group, using the same NIRS spatial configuration demonstrated similar findings in a marginally older cohort. Here, the impact of a task used to approximate naval warfare (requiring spatial and verbal working memory and decision-making processes), in the dorsolateral prefrontal cortex was assessed. Eight healthy subjects aged 18-50 years took part in the study. They observed (as anticipated based on the findings of their previous study) that as task difficulty increased so too did cerebral oxygenation, until the most difficult task where there was no further increase in oxy-Hb. Again it was concluded that the demands of a task can signal a change in oxygenation (Izzetoglu et al., 2004). Once again, no measurements of either deoxy-Hb or total-Hb were reported. Within the same study, it was also concluded that the rate of oxygenation change provides an index of sustained attention (in a complex working memory and decision-making task). This was demonstrated by a strong positive correlation between blood oxygenation and performance of the most difficult condition. This pattern whereby there is an increase in oxygenation as a result of increasing task difficulty was also observed by Shibuya-Tayoshi et al. (2007). In this study, 41 healthy participants aged 22–49 years (mean age 27.6 years) completed the trail-making task - a task of executive function, whilst prefrontal cortex activation was measured using NIRS, positioned across the forehead in a 22-channel configuration. This task is made up of two parts (A and B) with part A being easier than the more difficult part B. The main finding was that of a significantly larger increase in oxy-Hb during the more difficult part B than during part A of the task. No correlation was observed however between oxy-Hb and task performance and once again, no effects on deoxy-Hb or total-Hb were reported.

Studies where the effects on deoxy-Hb and total-Hb are reported (in addition to those on oxy-Hb), perhaps give a fuller picture of the effects of task performance and difficulty on cerebral oxygenation. Schroeter et al., (2002) measured haemodynamics using NIRS in the frontal cortex during neutral, congruent and incongruent trials of the Stroop task in 14 healthy young male participants aged 19-29 years (mean age 23.9 years). The results demonstrated that concentrations of oxy-Hb and total-Hb increased and deoxy-Hb decreased. Furthermore, the incongruent trials led to a stronger vascular

response than neutral trials, a finding which confirmed an increase in neuronal activity as a result of the interference caused by the (more difficult) incongruent version of the task. In an assessment of the haemodynamic impact of the Wisconsin Card Sorting task (WCST), Fallgatter and Strik, (1998) measured oxy-Hb and deoxy-Hb in the frontal cortex of 10 healthy young subjects aged 27-33 years (mean age 30 years). Their findings demonstrated a significant increase in oxy-Hb during performance on the WCST as compared to a resting baseline. A corresponding decrease in deoxy-Hb was also reported; however, this effect failed to reach significance. Medvedev et al., (2011) assessed haemodynamic change in the pre-frontal cortex using a 3 x 8 configuration across the inferior frontal gyrus. Eleven healthy participants aged 18-30 (mean age 23 years) were required to perform the object recognition task (Go-NoGo task). They observed an increase in oxy-Hb, with a corresponding decrease in deoxy-Hb that was found to be greater in the left versus the right hemisphere.

The evidence from functional NIRS studies during the performance of cognitive tasks would appear to suggest that cerebral activation as identified by fNIRS is expressed as an increase in oxy-Hb (and or total-Hb) and (in some instances) a corresponding decrease in deoxy-Hb. However, as will be discussed in the next section, this pattern of effects is subject to change with the introduction of nutritional or pharmacological interventions.

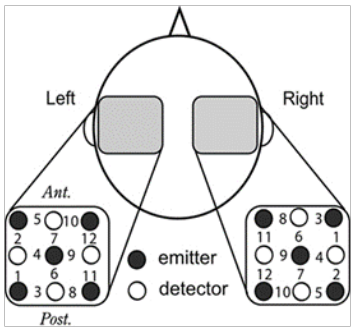


Figure 1.2 Example of arrangement of NIRS device optodes, showing a multi-channel, 5 x 4 (emitter by detector) 12-channel configuration across each hemisphere, taken from Sato et al (2011). Included for reference purposes to demonstrate a multi-channel system. For example of configuration used in present thesis, please see figure 1.1.

1.3.4.4 fNIRS and fMRI

Since fMRI is considered the current gold standard for functional neuroimaging, a number of studies have sought to evaluate the concurrent use of NIRS and fMRI methods in an attempt to validate the NIRS technique as well as attempting to overcome each modality's inherent limitations. With a view to confirm the response pattern as a result of functional activation, Kleinschmidt et al., (1996) mapped the effects of a finger tapping task during the simultaneous acquisition of MRI and NIRS measurements. Through exact spatial matching of the NIRS optodes to the task-activated motor cortex area (identified by MRI), they confirmed that both methods observed a decrease in deoxy-Hb, NIRS also documented an increase in oxy-Hb; however, this was reasoned to be smaller and less specific than that of deoxy-Hb. Cui et al., (2011) monitored simultaneous NIRS and fMRI measurements during the completion of a battery of cognitive tasks. Here it was observed that whilst both oxy-Hb and deoxy-Hb correlated with the fMRI BOLD signal, oxy-Hb demonstrated a statistically significant advantage over deoxy-Hb (albeit a small one). In other studies of NIRS and fMRI data acquisition, the simultaneous use of these systems has established that although NIRS has lower signal-to-noise ratio, NIRS measures of both deoxy-Hb (Huppert et al., 2006; Sato et al., 2013; Schroeter, Kupka, Mildner, Uludag, & Von Cramon, 2006; Toronov, Webb, & Choi, 2001) and oxy-Hb (Strangman, Culver, Thompson, & Boas, 2002) correlate well with the fMRI BOLD signal. Furthermore, Obrig et al. (2000) identified a decrease in deoxy-Hb during fNIRS studies as a robust indicator of haemodynamic change as a result cerebral activation.

1.3.4.5 fNIRS and EEG

There are an increasing number of studies that are using EEG in combination with NIRS in order to provide a concomitant assessment of cerebral activation. The benefit of this approach is that simultaneous measures of local haemodynamic and neuronal electrical activity can be obtained at a high temporal resolution. These measures can be achieved over lengthy periods of time, in a quiet setting, under conditions that more closely reflect everyday life, where participants are not immobilised (Wallois, Mahmoudzadeh, Patil, & Grebe, 2012). The integration of NIRS and EEG technologies

has been successfully adopted in children in the assessment of the new-born auditory cortex and its sensitivity to the temporal structure of sounds (Telkemeyer et al., 2009). It has also been applied to determine the relationship between spontaneous neuronal activity and cerebral haemodynamic processes in sleeping premature neonates (Roche-Labarbe, Wallois, Ponchel, Kongolo, & Grebe, 2007). In adults, co-registration of these two methods has been demonstrated during functional activation whilst responding to a simple checkerboard stimulus (Obrig et al., 2002)**,** performing the odd-ball task (Tong et al., 2005) and processing emotional stimuli (Herrmann et al., 2008). It has also been used in the assessment of brain computer interface research (Fazli et al., 2012), and during language processing (Ehlis et al., 2009; Wallois et al., 2012).

1.3.4.6 fNIRS and interventions of interest to cognition

Nutritional interventions, which have demonstrated positive effects on behaviour, may, in some cases, also be capable of augmenting cerebral haemodynamics and blood flow. It is this relationship between the observed behavioural and physiological cognitive response, which is of great interest, and one which requires additional research within the nutritional interventions field. Assessment of cerebral activation (using NIRS) as a result of cognitive performance following the administration of interventions of nutritional interest is an area that is receiving more attention in the literature. A number of studies have been conducted that have demonstrated changes in cerebral oxygenation using NIRS during task performance in the presence of a range of acute and chronic nutritional challenges.

Unless otherwise stated all of the following studies are double-blind, placebo-controlled, crossover studies.

1.3.4.6.1 fNIRS and caffeine

In one of the very first fNIRS interventional studies, Niioka and Sasaki, (2003) measured cerebral blood flow in the left prefrontal cortex using a 1 x 1 (1-channel) configuration. Following administration of placebo or 200 mg caffeine (a member of the methylxanthine family known for its vasoconstrictive effects), participants completed the Stroop task. Ten, healthy participants (no ages reported) took part and the findings demonstrated that caffeine significantly reduced oxy-Hb and total-Hb as compared to

placebo during performance of the Stroop task. No effects of caffeine on performance of the task were found, however. Similarly, Higashi et al., (2004) utilised NIRS to measure regional cerebral blood volume (rCBV) in the frontal cortex during a mentally demanding task following acute caffeine consumption. Here, 14 participants (21-50 years) completed the Uchida-Kraepelin psychodiagnostic (UKP) test (a serial addition test) at baseline and following 180 mg caffeine. Caffeine intake led to a significant reduction in rCBV as compared to placebo. A significant improvement in the number of correct answers was also observed following caffeine consumption, although this was not found to be coupled with the corresponding change in rCBV. There are, however, a number of limitations to both of these studies; firstly, the caffeine conditions were compared against 'caffeine-free' or decaffeinated coffee. Decaffeinated coffee cannot be regarded as a true caffeine free alternative as decaffeinated coffee still contains caffeine at levels shown to have psychoactive effects (Haskell, Kennedy, Milne, Wesnes, & Scholey, 2008a). In addition, coffee is a beverage that contains other psychoactive compounds (including polyphenols, which have been shown to increase CBF) therefore any effects observed may not be wholly attributable to the effects of caffeine alone. Furthermore, within both studies, the authors did not account for caffeine consumption status. This is an important factor when assessing the effects of caffeine, as although effects have been demonstrated in both consumers and non-consumers, differences exist between their response, both behaviourally (Haskell, Kennedy, Wesnes, & Scholey, 2005; Smit & Rogers, 2000) and physiologically in terms of blood flow (Addicott et al., 2009; Field, Laurienti, Yen, Burdette, & Moody, 2003; Kennedy & Haskell, 2011). Finally, in the latter study, the age range used (21-50 years) is very wide-ranging, particularly considering that CBF is thought to reduce with increasing age (Leenders et al., 1990; Martin et al., 1991; Pantano et al., 1984), a factor that may have impacted upon the results. In a more recent study, Heilbronner et al., (2015) assessed the CBF effects of caffeine during a working memory (2-back) task in 10 moderate caffeine consumers (19-34 years, mean age 29.1 years). Using a 3 x 11 (52 channel) NIRS configuration, they found that 200 mg caffeine led to a significant reduction in oxy-Hb in both the left and right hemisphere of the inferior frontal cortex, with a

corresponding increase in deoxy-Hb in the left hemisphere, alone. There were no effects of caffeine on behavioural performance. Although this study took caffeine consumption status into consideration, there were a number of other issues with the study that the authors themselves acknowledged. The study was not placebo controlled, nor was it double blind and the order in which participants completed the control and treatment sessions were not counterbalanced. Furthermore, participants were only restricted from specifically coffee intake on the morning of the study visit, not caffeine, and as it is well known, caffeine can be found in many sources other than coffee. In a more thorough investigation of the acute effects of caffeine, Kennedy and Haskell, (2011) used a 2 x 1 (2-channel) configuration on the frontal cortex, to examine the effects of 75 mg caffeine, in habitual and non-habitual caffeine consumers. Twenty, healthy young adults (mean age 21.4 years; 10 habitual consumers and 10 non-habitual consumers) completed the CDB. As previously discussed, the CDB is a series of cognitively demanding tasks that have been shown to be sensitive to nutritional interventions. It was demonstrated that 75 mg caffeine led to a significant reduction in total-Hb during cognitive task performance as compared to placebo; however, this effect was only observed in non-habitual consumers. Despite this isolated effect of blood flow, behavioural effects were demonstrated across groups, where caffeine led to a significant reduction in the number of serial 7s subtractions errors as compared to placebo, irrespective of consumer status. A consistent pattern within the aforementioned fNIRS studies where caffeine is administered, is that the reductions in oxy-Hb and or total-Hb (CBF), or changes in deoxy-Hb, do not negatively impact upon behavioural performance. The relevance and mechanism of this effect will be discussed later. However, observations such as this - where there is a change in cerebral oxygenation during task performance that does not translate to positive or negative behavioural effects has also been observed following other interventions.

1.3.4.6.2 fNIRS and polyphenols

Using the same 2-channel NIRS configuration to interrogate the pre-frontal cortex as described by Kennedy and Haskell (2011), Wightman et al., (2012) assessed the effects of 2 acute doses (135 mg and 270 mg) of epigallocatechin gallate (EGCG). A

polyphenol found in the tea plant, EGCG is thought to confer a number of health benefits, including modulation of blood flow and brain function. Twenty-seven healthy young adults (mean age 22 years), performed a range of cognitive tasks whilst haemodynamics were monitored via NIRS. The results demonstrated that EGCG led to a significant decrease in total-Hb and oxy-Hb during the task period following the lower (135 mg) dose as compared to placebo, in the absence of any change in behavioural performance. In an attempt to delineate the cerebrovascular effects of the polyphenol and vasodilator resveratrol, Kennedy et al., (2010) employed NIRS to detect oxygenation changes in the frontal cortex using the same configuration and during completion of the same tasks as described by Kennedy and Haskell (2011). Following administration of doses of 250 mg and 500 mg, they observed that the 500 mg dose of resveratrol led to significant increases in total-Hb and deoxy-Hb as compared to placebo in healthy young adults (mean age 20.2 years). They identified this finding as indicative of increased blood flow and oxygen extraction, in the absence of any effects on behavioural performance. In a further study of 23 healthy young adults, aged 19-34 (mean 21 years) using the same design (with additional tasks), 250 mg resveratrol was administered alone and in combination with 20 mg piperine. It was anticipated that this alkaloid would enhance the bioavailability and bioefficacy of resveratrol. Following co-administration with piperine, resveratrol led to a significant increase in oxy-Hb, deoxy-Hb and total-Hb as compared to placebo and resveratrol alone, during the performance of cognitive tasks. However, once again, no concomitant effects of treatment on cognitive performance were observed (Wightman et al., 2014). A chronic investigation of resveratrol was conducted by the same group, using the same NIRS configuration and similar tasks. Here a 500 mg dose administered to 60 healthy adults aged 18-30 years (parallel groups design), failed to modulate cerebral haemodynamics following 28 days' supplementation. However, the initial, acute dose, led to an increase in oxy-Hb and total-Hb and, in contrast to previous studies, a reduction in deoxy-Hb. In this study there were also findings in relation to behaviour; however, they were not consistent in terms of their direction and the authors concluded they should be treated with caution (Wightman, Haskell-Ramsay, Reay, et al., 2015). Again, it is of

interest here that despite the augmentation of oxy-Hb as well as total-Hb (and in some cases deoxy-Hb), there were few or no concomitant improvements in behavioural performance across these studies.

1.3.4.6.3 fNIRS and fish oil

Jackson et al., (2012a) assessed the impact of chronic supplementation of omega-3 polyunsaturated fatty acid (PUFA) on cerebral oxygenation, using the same 2-channel configuration as Kennedy and Haskell (2011). In an independent measures study, 22 healthy young adults (mean age 21.9 years) received 12 weeks' daily supplementation of 1 g docosahexaenoic acid (DHA)-rich fish oil, 1 g eicosapentaenoic (EPA)-rich fish oil or placebo (olive oil). Chronic supplementation with 1 g DHA-rich fish oil led to a significant increase in oxy-Hb during the Stroop task, and a significant increase in total-Hb during the Stroop, peg and ball and 3-back tasks as compared to placebo. There were, however, no associated effects upon cognition. In a further study, Jackson, Reay, Scholey, and Kennedy (2012b) administered 1 g or 2 g of DHA-rich fish oil or placebo for 12 weeks to 65 healthy adults (mean age 20.6 years) whilst cerebral oxygenation was measured in the pre-frontal cortex (PFC) (as above), during cognitive tasks. They observed that both doses led to a significant increase in oxy-Hb during the entire testing battery (which included a range of memory, attention and executive function tasks). For total-Hb the same consistent pattern of significant effects was observed across tasks for the 2 g dose; however, following the 1 g dose, it was only observed during two of the cognitive tasks, suggesting a dose-response effect for total-Hb. There were no effects of either dose on deoxy-Hb. There was some evidence of modulation of behavioural performance on reaction time and a task of attention; however, the authors note they should be treated with caution, since, if corrections for multiple testing had been conducted, these significant effects would have been lost. NIRS has also been used to measure changes in oxygenation status following chronic administration of PUFAs in healthy elderly adults. Using an independent measures design, Konagai et al., (2013) administered either 2 g krill oil (an omega-3 PUFA-rich plankton oil), sardine oil (also high in omega-3 PUFAs) or a placebo (containing medium-chain triglycerides) to 42 healthy older males aged 61-72

(mean age 67.1 years) every day for 12 weeks. Performance on a version of the 2-back working memory task was assessed via simultaneous fNIRS and EEG and performance of the UKP task was assessed via fNIRS following 6 and 12 weeks' supplementation. A 24-channel array measured NIRS readings from the dorsolateral pre-frontal cortex. It was found that 12 weeks' supplementation of both krill and sardine oil led to significantly elevated levels of oxy-Hb as compared to placebo during the 2-back task. Krill oil alone significantly increased oxy-Hb as compared to placebo during the UKP test. Krill oil also resulted in a significant reduction in P300 latency at Cz and Pz sites as compared to placebo, following 12 weeks' supplementation. Unfortunately, no details were provided regarding performance on either of the cognitive tasks so it is not possible to draw any conclusions on whether the observed increases in oxy-Hb or the reduction in P300 latency translated into positive or negative behavioural effects, or if there was an absence of an effect altogether.

1.3.4.6.4 fNIRS and other nutritional interventions

In a further chronic study, Konagai et al., (2013) assessed cerebral oxygenation following 7 days supplementation with essence of chicken extract. Essence of chicken (EOC) is a traditional protein, peptide and amino acid containing drink, consumed regularly in Southeast Asia as a nutritional supplement and as a treatment for a number of ailments, including anxiety. Twelve healthy older participants, aged 60-68 (mean age 62.3 years) were assessed during the performance of tasks assessing working memory, spatial memory and reaction time. fNIRS readings were recorded from the pre-frontal cortex using a 25-channel configuration. It was demonstrated that compared to baseline performance of the 2-back task, oxy-Hb was significantly elevated following 7 days' supplementation with EOC. In contrast, there were no effects following placebo and there were no concomitant effects on behavioural performance. Unfortunately, no comparison of active treatment to placebo was made within this study. In an evaluation of the chronic effects of creatine, Wantanabe et al, (2002) assessed cerebral oxygenation following administration of 8 mg/day for 5 days during performance of the UKP task and the level of fatigue elicited as a result. As compared to pre-treatment task performance, creatine led

to a significant reduction in oxy-Hb and a significant increase in deoxy-Hb, in addition to a reduction in mental fatigue. However, again, despite a placebo condition being tested, no comparisons of active treatment were made against placebo within this study. Bonoczk et al., (2002) used NIRS in conjunction with transcranial Doppler (TCD) to investigate cerebral blood flow and oxygenation changes in stroke patients following the intravenous administration of vinpocetine. Vinpocetine is a vinca alkaloid, derived from the periwinkle plant, which is capable of increasing microcirculation and cerebral oxygen uptake. In an independent measures study, ischemic stroke patients (mean age 65 years) were administered either 20 mg vinpocetine or placebo. NIRS readings were taken at rest, fronto-laterally on the lesional side, with TCD readings taken from the middle cerebral artery (MCA) on the lesional side. The results demonstrated that vinpocetine infusion led to a significant increase in deoxy-Hb and a significant increase in Doppler spectral intensity (DSI) as compared to placebo, with the authors concluding that these findings are indicative of increased regional cerebral blood flow and oxygen extraction.

Based on the fNIRS studies presented here it would be fair to surmise that modulation of cerebral oxygenation as a result of nutritional challenge, does not necessarily predict the presence or absence of a behavioural effect. The interaction of these factors is clearly complex and requires further research.

1.4 Nutritional interventions of cognitive interest

There is a wealth of research that has been conducted assessing the cognitive benefits that nutritional interventions can convey. Intervention-related cognitive research tends to focus on the behavioural effects of interventions, through the use of testing batteries or via tasks specific to relevant cognitive domains (memory, attention etc.); however, some also take into account measures of physiological effects. Examples of interventions that have received a lot of interest in terms of their effects on cognition are detailed in the following section, with a spotlight on those that have an impact on cognition in young, healthy adults and the ability to modulate neurophysiological parameters.

1.4.1 Interventions of interest to cognition

1.4.1.1 Herbal extracts

The use of herbal extracts for the improvement of health and wellbeing has gained increased interest in more recent times, with people looking for seemingly more 'natural' methods where they can self-medicate in order to benefit health. Of the extracts that have received attention, two examples have received a degree of interest in terms of research for their purported cognitive enhancing effects; *Panax ginseng* and *Ginkgo biloba*.

1.4.1.1.1 Ginkgo biloba

Ginkgo biloba is an extract taken from the leaves of the G*inkgo biloba* (or maiden hair) tree, used for centuries within Chinese medicine for a range of ailments. *Ginkgo biloba* belongs to the Ginkgoceae botanical family and the ginkgo tree from which the extract is obtained is one of the oldest living species on the planet (Mahadevan & Park, 2008). It has received attention more recently, as a therapy capable of modulating 'cerebral insufficiency' or memory impairment in older populations (Kleijnen & Knipschild, 1992), as a potential treatment for dementia (LeBars et al., 1997) and for augmenting cerebral blood flow (Mashayekh et al., 2011). Although at present, the specific mechanisms by which ginkgo augments peripheral and cognitive parameters are not fully understood, biflavone glycosides and terpene lactones are the active constituents within ginkgo that are thought to underlie its effects (Park, Rhee, & Lee, 2005). In-*vivo* and in-*vitro* assessments of these fractions have demonstrated that their administration leads to a number of effects that may explain some of ginkgo's therapeutic actions, for example platelet activating factor antagonism (Koch, 2005; Stromgaard et al., 2002), implicated as a mechanism by which ginkgo may exert its neuroprotective action (Smith, Maclennan, & Darlington, 1996). These fractions have also led to modulation of neurotransmitters, for example through the increased uptake of serotonin (Ramassamy, Christen, Clostre, & Costentin, 1992). Increases in dopamine and noradrenaline release have also been observed, which may relate to improvements in cognitive function (Kehr et al., 2012). Increased nitric oxide production via endothelial nitric oxide synthase which may lead to vasodilation (Ahlemeyer & Krieglstein, 2003). It also possesses antioxidant properties

(Bridi, Crossetti, Steffen, & Henriques, 2001; Maitra, Marcocci, Droylefaix, & Packer, 1995).

The methodological approach adopted by studies assessing the cognition-related efficacy of ginkgo in older populations, has focussed on the cognitive task related effects of ginkgo as a primary outcome measure, specifically tasks that assess learning and memory (Gold, Cahill, & Wenk, 2002). Recent reviews of ginkgo's efficacy within clinical populations have determined that in the treatment of Alzheimer's disease (AD) ginkgo performs better than placebo and is able to help with the cognitive symptoms of sufferers of established AD (Weinmann, Roll, Schwarzbach, Vauth, & Willich, 2010; Yang et al., 2014); although it does not prevent the disease's neurodegenerative progression (Yang et al., 2014). Other approaches in the evaluation of ginkgo's cognitive effects in older populations have included EEG assessments of cerebro-electrical activity. Here ginkgo has been shown to reduce P300 latency in patients with age associated memory impairments (AAMI) (Semlitsch, Anderer, Saletu, Binder, & Decker, 1995). It has also led to modulation of theta and alpha activity in dementia sufferers (Hofferberth, 1994; Itil, Eralp, Ahmed, Kunitz, & Itil, 1998) as well as in healthy older participants (Gessner, Voelp, & Klasser, 1985; Itil, Itil, Eralp, & Le Bars, 1996). Research of the extracts' vascular effects, particularly in terms of CBF, includes only a limited number of studies. Assessments using dynamic susceptibility contrast-enhanced magnetic resonance imaging and Single Photon Emission Computer Tomography have demonstrated that in healthy older participants, chronic ginkgo administration leads to increased CBF (Mashayekh et al., 2011; Santos et al., 2003). One study to date has assessed the chronic effects of ginkgo on blood oxygenation using NIRS. The study found that reaction time on a working memory task was improved in middle aged women (44-55) and that the pattern of PFC activation was changed to right-dominant, following 6 weeks of 120 mg/day gingko supplementation (Sakatani, Tanida, Hirao, & Takemura, 2014)

Within healthy young populations, ginkgo research has also tended to focus on the cognitive-task related effects of the extract, with few studies employing additional methods

of evaluation such as neuroimaging, to investigate neurophysiological correlates. Assessments of cognitive ability have, in the majority of studies, been focussed on tasks of attention and memory, with largely positive outcomes being observed as a result of ginkgo administration (Elsabagh, Hartley, Ali, Williamson, & File, 2005; Kennedy, Jackson, Haskell, & Scholey, 2007; Kennedy, Scholey, & Wesnes, 2000, 2002; Stough, Clarke, Lloyd, & Nathan, 2001). The limited number of studies that have adopted other methods of assessment have utilised EEG to monitor cerebro-electrical activity within this population. However, although modulation of activity has been observed following ginkgo administration across all EEG studies, the findings are relatively inconsistent overall (Itil et al., 1996; Kennedy et al., 2003; Kunkel, 1993).

1.4.1.1.2 Panax ginseng

The ginseng plant belongs to the Araliaceae family; *Panax ginseng* is one of 13 species of ginseng and is grown in the USA, Russia and most notably the Far East where it has been used for thousands of years as a medicinal herb (Yun, 2001). *Panax ginseng* is an extract sourced from the root of the ginseng plant and is a herbal remedy which has had a long history of medicinal use in a number of countries for its ability as a tonic. In this respect it has been used to strengthen and invigorate a weakened body and to alleviate symptoms including headache, fatigue, dizziness, nausea, haemorrhage and impotence (Park et al., 2005). The active constituents within ginseng are saponins or ginsenosides, with the most prevalent in *Panax ginseng* being Rg1 and Rb1 (Sengupta et al., 2004). The main focus of ginseng research in humans has been upon its potential to modulate cognition. Within clinical populations, ginseng has been shown to enhance cognitive performance in both AD patients (Lee, Chu, Sim, Heo, & Kim, 2008) and those with age, associated memory impairments (AAMI) (Neri, Andermarcher, Pradelli, & Salvioli, 1995). However, reviews of the extracts' efficacy on neurodegenerative diseases have reported that evidence for its use as an AD treatment is scarce and inconclusive (Lee et al., 2008) and there is a lack of high quality evidence of its effects in dementia (Geng et al., 2010). Within younger populations, a more exploratory approach has been adopted to identify behavioural effects of the extract. Studies have employed a range of

tasks assessing attention, reaction time, executive function, memory and mood and these studies have obtained mixed results in terms of ginsengs' efficacy following acute and chronic supplementation (Dangelo et al., 1986; Kennedy, Haskell, Wesnes, & Scholey, 2004; Kennedy, Scholey, & Wesnes, 2001b; Kennedy et al., 2002; Reay et al., 2005, 2006; Sunram-Lea, Birchall, Wesnes, & Petrini, 2005). Despite the interest in ginsengs' effects on cognitive performance, human studies assessing the associated neurophysiological effects of the extract are few, with only one study to date evaluating its cerebro-electrical effects using EEG (Kennedy et al., 2003).

1.4.1.2 Caffeine

A member of the methylxanthine family and a substance that is found to occur naturally in plants, caffeine is another example of a nutritional intervention that is able to convey cognitive benefits. The mechanism by which caffeine exerts its effects is now largely accepted to be through antagonism of adenosine A_1 and A_{2A} receptors (Fredholm, Battig, Holmen, Nehlig, & Zvartau, 1999a), with antagonism of A_1 effects being more closely related to neural activation and A_{2A} antagonism resulting in vascular effects (Laurienti et al., 2003). There is an abundance of research assessing caffeine's behavioural effects on cognition, and its ability to improve subjective alertness and performance on tasks of attention are well documented (Childs & de Wit, 2006; Haskell, Kennedy, Milne, Wesnes, & Scholey, 2008b; Quinlan et al., 2000; Rogers, 2007; Smit & Rogers, 2000). Findings such as these have perhaps contributed to its reputation for being the most widely consumed psychoactive substance in the world (Fredholm et al., 1999a). Caffeine is also known for its vascular effects and studies have documented both elevations in blood pressure (Noordzij et al., 2005; Nurminen, Niittynen, Korpela, & Vapaatalo, 1999; Rogers, Smith, Heatherley, & Pleydell-Pearce, 2008) as well as constricting effects on the cerebral vasculature. Magnetic resonance imaging (Addicott et al., 2009; Chen & Parrish, 2009a; Field et al., 2003; Haller et al., 2013; Laurienti et al., 2003; Mathew & Wilson, 1991; Rack-Gomer, Liau, & Liu, 2009) as well as NIRS studies (Kennedy & Haskell, 2011) have demonstrated that caffeine significantly reduces cerebral blood flow. Reductions in cerebral blood velocity have also been observed, as assessed

by transcranial Doppler (Hasse et al 2005). Interventional studies that have had a cognitive focus have therefore adopted measurements of peripheral and or central physiological parameters during caffeine's assessment (Haller et al., 2013; Kennedy & Haskell, 2011; Rogers et al., 2008). One way in which caffeine could be termed unique in comparison to many other nutritional interventions, is in its capacity to uncouple blood flow. Calibrated to hypercapnia BOLD fMRI studies have demonstrated that following a ~200 mg dose of caffeine, CBF is reduced; however, the cerebral metabolic rate of oxygen ($CMRO_2$) remains unchanged (Perthen, Lansing, Liau, Liu, & Buxton, 2008), or increases in response to task stimulation (Chen & Parrish, 2009a). For this reason its proposed use as a BOLD contrast booster in MRI studies (Mulderink, Gitelman, Mesulam, & Parrish, 2002) is controversial (Laurienti et al., 2003). Owing to the way in which BOLD is measured (being sensitive to changes in the level of oxygenation as opposed to changes in blood flow directly), fMRI is not without its limitations in terms of the assessment of caffeine's cerebro-vascular and neural effects, despite being the gold standard for neuro-imaging.

1.4.1.3 L-theanine

A naturally occurring amino-acid, L-theanine is found almost uniquely in tea *(Camellia sinensis*), where it coexists with caffeine. L-theanine has a chemical structure similar to that of the neurotransmitter glutamic acid (Nathan, Lu, Gray, & Oliver, 2006) and is able to cross the blood-brain barrier. It also has the ability to alter neurotransmitter concentrations; increases in dopamine concentrations have been observed in the rat brain in a dose dependent manner (Yokogoshi, Kobayashi, Mochizuki, & Terashima, 1998), intraperitoneal administration to mice led to an increase in GABA (Kimura & Murata, 1971) and modulation of serotonin levels in rats have also been observed (Yokogoshi et al., 1995; Yokogoshi et al., 1998). L-theanine has also been shown to protect against human neuronal cell death *in vitro* (Cho et al., 2008). Interventional studies that have investigated its capacity as an anxiolytic have demonstrated positive effects (Kimura, Ozeki, Juneja, & Ohira, 2007; Rogers et al., 2008). However, it has received most attention within human research for its cognitive effects, both alone and in combination

with caffeine (Gomez-Ramirez et al., 2007; Gomez-Ramirez, Kelly, Montesi, & Foxe, 2009; Haskell et al., 2008a; Kelly, Gomez-Ramirez, Montesi, & Foxe, 2008; Owen, Parnell, De Bruin, & Rycroft, 2008). In terms of its neurophysiological effects, although there have been assessments of cerebro-electrical parameters as measured by EEG (Gomez-Ramirez et al., 2007; Gomez-Ramirez et al., 2009; Juneja, Chu, Okubo, Nagato, & Yokogoshi, 1999; Nobre, Rao, & Owen, 2008), no studies to date have used alternative neuro-imaging techniques to measure the effects of L-theanine administration. In addition, where cerebro-electrical activity and behaviour have been co-monitored, all studies have focussed on the effects during performance of predominantly attentional tasks (Gomez-Ramirez et al., 2007; Gomez-Ramirez et al., 2009; Kelly et al., 2008).

1.4.1.4 Beetroot

Beetroot is a member of the *chenopodiaceae* family and a vegetable receiving an increased level of interest within exercise and cognition-related studies, due to the comparatively high levels of nitrate it contains (Santamaria, Elia, Serio, & Todaro, 1999). The significance of this being that supplementary dietary nitrate has been associated with increased nitric oxide (NO) production (Webb et al., 2008). Nitric oxide is a signalling molecule which, amongst other actions, is known to modulate cerebral and peripheral vasodilation (Moncada & Higgs, 1993; Toda, Ayajiki, & Okamura, 2009). The majority of studies to date that have evaluated the effects of beetroot juice, have had a methodological focus that is primarily exercise-related (Bailey et al., 2010; Bailey et al., 2009; Bond et al., 2013; Kelly et al., 2013; Kenjale et al., 2011; Lansley, Winyard, Bailey, et al., 2011; Lansley, Winyard, Fulford, et al., 2011; Vanhatalo et al., 2010). Those that have included cognitive tasks, have either done so in the absence of exercise (Wightman, Haskell-Ramsay, Thompson, et al., 2015), or have conducted them post-exercise, (Kelly et al., 2013), with only two during exercise performance (Rattray et al., 2015; Thompson et al., 2015). Of these two studies, only one simultaneously measured cerebral perfusion during performance of the tasks (Rattray et al., 2015). Where neurophysiological assessments are concerned therefore, there are few studies that have explored the

effects of beetroot in healthy, young humans during cognitive and simultaneous exercise performance.

1.5 General conclusions

The identification of nutritional interventions with the capability to improve cognitive performance or protect against cognitive decline is of increasing importance, particularly in light of an ageing population. This, coupled with a heightened awareness of the potential for nutritional supplementation to improve our cognitive as well as our physiological health has meant that now, more than ever, public interest in this area is high. This is demonstrated by a rise in the availability and sale of off-the-shelf remedies with a concomitant increase in self-medication. Indeed in the UK in 2009, the dietary supplements and vitamins market was worth at least 670 million, representing a growth of over 16 % between 2006 and 2011 (NHS, 2011). Patterns of behaviour such as this highlight the need for ongoing research into the effects of nutritional interventions and the benefits they may convey.

The methods and technologies available to monitor the cognitive effects of nutritional interventions are wide-ranging; however, not all lend themselves to being suitable for integration with other technologies and methodologies. The benefit of adopting such an approach is that it is possible to obtain information not only in relation to the cognitive effects the supplement can convey but also perhaps the physiological means by which these effects occur. NIRS has established itself as a valid method for the measurement of cerebral oxygenation at rest and during the performance of cognitive tasks as well during active states and whilst exercising and also one that can be incorporated with other imaging modalities (for example fMRI and EEG).

1.6 Summary and objectives of the thesis

An increase in the availability of new technologies has meant that novel approaches for testing cognitive ability can be broached. In conjunction, a growing

flexibility in the ability of imaging techniques such as NIRS to assess neurophysiological parameters under a variety of conditions means that there is now scope for assessing the impact of nutritional interventions within a range of paradigms. There are a number of nutritional interventions known to modulate cerebral blood flow, examples of which include caffeine, L-theanine, ginkgo, ginseng and beetroot juice (nitrates). This ability, coupled with the fact that each is also known to possess cognition enhancing properties means they are ideally suited for use within studies assessing not only behavioural but also neuro-physiological performance.

The main aims of the thesis are:

- The identification of technologies that can be used either together (or alone) in the novel assessment of nutritional interventions and their effects upon cognition.
- To identify methodologies that can be implemented concomitantly during the assessment of interventions of interest to cognition.
- To expand the current knowledge base on the ability of specific nutritional supplements to benefit cognitive and physiological performance.

Chapter 2. The effects of *Ginkgo biloba* and a *Ginkgo biloba/Panax ginseng* combination on cerebro-electrical activity and blood flow in healthy adults.

2.1 Introduction

Ginkgo biloba is currently marketed as a herbal remedy capable of enhancing cerebral blood flow and improving memory (Smith et al., 1996). It is beyond the scope of this thesis to review all studies relating to ginkgo and its effects, therefore only the most pertinent in terms of those that have assessed behavioural or neurophysiological effects or a combination of these factors are discussed below. In terms of behaviour, there have been a number of studies that have assessed the effects of an acute, single dose of ginkgo, (standardised to approximately 24% flavone glycosides, 6% terpene lactones) the majority of which have been conducted within healthy young populations. Elsabagh et al. (2005) observed that an acute dose of 120 mg ginkgo led to an improvement in sustained attention. Although this same dose has since been found to reduce the speed of performing attentional tasks (Kennedy, Jackson, et al., 2007), higher doses of 240 mg and 360 mg have been found to significantly increase in the speed of performing attentional tasks, 2.5, 4 and 6 hours post treatment (Kennedy et al., 2000). Enhanced performance on tasks of mental arithmetic has also been observed following a 360 mg dose, as demonstrated by a reduction in the number of errors made on a serial 3s subtraction task and an increase in total serial 7s subtractions responses (Kennedy et al., 2002). Acute administration has also led to improvements in memory as assessed by a 'quality of memory' factor following doses of 120 mg and 360 mg (Kennedy, Jackson, et al., 2007; Kennedy et al., 2002). Turning to chronic effects, again, there have been a number of studies assessing the behavioural impact of ginkgo supplementation; however here, a greater proportion of research has been conducted in older participants, in addition to healthy young populations. Stough et al. (2001) observed that 30 days supplementation with 120 mg of ginkgo to healthy, young participants resulted in significant improvements in speed of working memory (Digit Span backwards) and memory consolidation (Auditory

Verbal Learning Test). However, Elsabagh et al. (2005) found that the same dose, administered for 6 weeks (42 days) did not lead to any improvements in tasks measuring sustained attention, memory or mood. Other studies assessing the behavioural effects of chronic ginkgo supplementation in healthy, older cohorts have shown that administration of 80 mg/day for 14 days resulted in a significant increase in accuracy on an object working memory task (Silberstein et al., 2011). Mix & Crews, (2000) observed that 6 weeks administration of 180 mg/day led to a significant improvement in performance on the Stroop task. A further study by the same group identified that and identical dose of ginkgo over the same duration led to an increase in the recall and recognition of auditory-verbal material on the Selective Reminding Test (SRT) (Mix & Crews, 2002). A longer, 8 month intervention with 80 mg/day ginkgo resulted in improvements on a number of tasks assessing attention, information processing speed and visuospatial ability (Santos et al., 2003). In summary, the evidence presented here, appears to suggest that overall acute supplementation with ginkgo in healthy young adults may lead to improvements in mental arithmetic, memory and attention, with improvements in speed of performing attentional tasks being observed following higher doses. The evidence in relation to chronic supplementation also appears to reflect an encouraging pattern of effects in older populations; leading to improvements in memory, attention and executive function. However, in young adults, the findings are less consistent and although improvements have been observed in memory, an absence of effects in memory as well as other areas such as attention and mood have also been reported.

In relation to the neurophysiological effects of ginkgo, there is a relative absence of human research into the impact on cerebral blood flow (CBF) following acute administration, despite evidence that its antioxidant effects and those on nitric oxide (NO) synthesis may underlie the increases in CBF seen in animal studies (Ahlemeyer & Krieglstein, 2003). Where chronic administration has been assessed, most studies have focussed on healthy, older adults. Ginkgo administered to a cognitively intact cohort, aged 60-70 years at 80 mg/day for 8 months led to an increase in cerebral perfusion in frontal, parietal and occipital areas of the brain as assessed by Single Photon Emission

Computer Tomography (SPECT) (Santos et al., 2003). However, in a study of a similar cohort (cognitively intact, aged 51-71), the effects on CBF (as assessed by dynamic susceptibility contrast-enhanced magnetic resonance imaging) of 120 mg/day ginkgo over 4 weeks, were less convincing. Here a significant increase in the left parieto-occipital region was demonstrated; however, the increase was small and observed only when an uncorrected p-value ($p<0.001$) was applied (Mashayekh et al., 2011). It is also of note here that sample size was small (n=9) and the duration of supplementation was much shorter than that of Santos et al. (2003). In a study of pre-frontal cortex (PFC) activation, (using a larger sample, n=19), Sakatani et al. (2014) assessed the effects of 6 weeks supplementation with 120 mg ginkgo, in a healthy middle aged cohort (44-50 years) using NIRS. They demonstrated that during performance of the Sternberg working memory test, ginkgo led to a shift from bilateral activation (observed during performance of the task in the absence of treatment) to right-dominant PFC activation. Assessments of cerebro-electrical effects of the extract have used EEG to monitor brain activity, with the majority of studies focussing on healthy older populations or those with age-associated cognitive impairment. Acute administration in healthy populations (aged 18-65 years) has led to increases in alpha activity following 40 mg, 120 mg and 240 mg with effects being demonstrated 1 hour post-dose and continuing until 7 hours post-dose (Itil et al., 1996). A chronic dose (57 days) of 120 mg also led to shortened P300 latency in participants with age-associated memory impairment (Semlitsch et al., 1995), and a single 240 mg dose resulted in increases in resting alpha and a reduction in slow waves in dementia patients (Itil et al., 1998). Where cerebro-electrical activity has been assessed following chronic supplementation in healthy older adults (50-61 year olds), 80 mg/day for 14 days, led to an increase in amplitude and latency of steady state visually evoked potentials (SSVEP) during an object working memory task. Taken in conjunction with improved behavioural performance, this finding was identified as being representative of more efficient processing (Silberstein et al., 2011). In healthy older adults (aged 57-77 years) who reported some symptoms of cerebral deterioration and who had the weakest level of vigilance at baseline, 120 mg/day (administered for 4, 8 and 12 weeks) led to an increase

in resting alpha activity and an improvement in simple reaction times (Gessner et al., 1985). Where pathological cohorts have been used, administration of 120 mg for 57 days led to shortened P300 latency in participants with age-associated memory impairment (Semlitsch et al., 1995). In Alzheimer's dementia patients, 240 mg/day for 3 months led to a reduction in theta wave activity (Hofferberth, 1994). Taken together the research to date suggests that ginkgo is capable of positively modulating CBF and cerebro-electrical activity; however, this is dependent upon dose and duration of supplementation.

Panax Ginseng (G115 extract, standardised to contain 4% ginsenosides) has been evaluated in a number of exploratory behavioural studies within young healthy populations. The findings have demonstrated largely positive effects during assessments of memory, attention and mood (Kennedy et al., 2004; Kennedy et al., 2001b, 2002; Reay et al., 2005; Reay, Scholey, & Kennedy, 2010; Sunram-Lea et al., 2005). Two hundred milligrams ginseng led to improved performance on a mentally demanding task (serial 7s subtractions) and reduced subjective feelings of mental fatigue (Reay et al., 2005) and both a 200 mg and 400 mg dose increased subjective feelings of calmness in healthy young adults (Reay et al., 2010). It has also been shown to improve secondary as well as working memory performance following acute supplementation of 200 mg (Kennedy et al., 2004) and 400 mg (Kennedy et al., 2001b, 2002; Reay et al., 2010) doses. However, the 200 mg dose has been shown to slow reaction time on a working memory task (Reay et al., 2010) and been associated with decrements in performance on a speed of attention factor (Kennedy et al., 2001b). However, this effect on speed of attention was not replicated in a further study with a similar methodology by the same research group (Kennedy et al., 2002) and was contrary to the findings of a later study, which demonstrated that 200 mg ginseng led to a significant improvement in speed of attention at both 4 and 6 hours post-dose (Kennedy et al., 2004). Similarly, a 400 mg dose of ginseng has also led to improvements in a speed of attention factor (Sunram-Lea et al., 2005). However, despite the positive effects on cognition, both a 200 mg and 400 mg dose have resulted in significant reductions in subjective alertness, six hours following treatment administration (Kennedy et al., 2001b). Despite the attention ginseng has

received in terms of its cognitive effects, there is an absence of human research into its effects on cerebral blood flow, with the only animal study to date demonstrating an increase in resting cerebral blood flow as assessed by laser-doppler flowmetry, 30 minutes after administration of 100 mg/kg of the crude saponin fraction of ginseng, to rats (Kim et al., 2002).

Turning to the combined effects of these extracts, the majority of human research conducted has also focused upon the effects on cognition. A study assessed three different acute doses of ginkgo/ginseng in combination (extracts standardised as previously reported) (320 mg combination = 120 mg ginkgo and 200 mg ginseng; 640 mg combination = 240 mg ginkgo and 400 mg ginseng; 960 mg combination = 360 mg ginkgo and 600 mg ginseng), in healthy young adults. They found that 960 mg led to a significant improvement in memory, with the lower two doses leading to a significant reduction in speed of attention (Kennedy, Scholey, & Wesnes, 2001a). A follow-up acute study in healthy young adults by the same research group assessing the highest 960 mg dose confirmed the effects on memory and also demonstrated a significant increase in the number of serial 3s and 7s subtraction responses made and a reduction in the number of serial 7s errors. Participants also rated themselves as being more content. However, a reduction in speed of attention was also reported at this dose (Kennedy et al., 2002). Findings across both of these studies were demonstrated between 1 and 6 hours post-dose. There are comparatively few studies assessing the behavioural effects of this combination, and with some inconsistencies in effects of those that have, there is evidently more scope for research in terms of the cognitive effects of this combination.

Despite the majority of research focussing on the effects on cognitive performance, one study has assessed the effects of this combination on cerebro-electrical activity via EEG. Dimpfel et al. (2006) administered a soft drink containing fruit juices and a combination of 114 mg ginseng and 980 mg ginkgo for 3 days to healthy participants aged 33-55 years (as compared to a matched placebo). They observed that the combination led to a significant increase in eyes-open delta power at rest and whilst reading a text in

central, parietal and occipital regions 2 hours after treatment, with a significant increase in theta power also observed whilst reading a text, 1 hour after treatment. In an attempt to delineate the acute cerebro-electrical effects of these individual herbal extracts, Kennedy et al. (2003) administered separate doses of 360 mg ginkgo and 200 mg ginseng to healthy young volunteers and measured EEG activity during resting eyes open/closed and whilst completing an auditory version of the oddball task. It was demonstrated that both ginkgo and ginseng led to a decrease in resting eyes closed theta and beta activity in the frontal region of the brain. Ginseng also led to a decrease in alpha activity and a reduction in P300 latency 4 hours post-treatment in left temporal and occipital brain regions. Overall, it was tentatively concluded that the EEG effects of ginseng were stronger than those of ginkgo, at the doses tested. The ability of ginkgo, ginseng, and their combination to modulate cerebro-electrical activity has been demonstrated; however, once again, the findings are not wholly consistent and therefore require further investigation.

With this in mind, the present study looked to extend this research and further explore the cerebro-electrical (EEG) activity of standardised (see treatment section of methodology) ginkgo and ginseng extracts, at rest and during task performance. Administration of a 207 mg dose of ginkgo, alone and in combination with a 207 mg dose of ginseng will be assessed following acute and chronic supplementation. The haemodynamic response to task performance will also be monitored (using NIRS) in order to explore the impact of these extracts during behavioural performance.

An extension of this research to include an exploration of the combination of these extracts was deemed necessary as previous research is limited to primarily behavioural outcomes. The only study looking at the effects of this combination on brain activity (via EEG), assessed the sub-acute (3 days) effects and used what appear to be unstandardised extracts and doses of ginkgo considerably larger than those used historically (Dimpfel et al., 2006). No studies to date have used EEG to assess the chronic effects of a ginkgo/ginseng combination on brain activity. In terms of cerebral

oxygenation, there is a complete absence of research into the effects of this combination, following acute or chronic administration across both young and older populations. The inclusion of NIRS will provide a direct measure of task-related cerebral haemodynamics and elucidate the effects of such a combination during behavioural performance. Despite ginkgo's historical reputation as an extract capable of improving CBF, human research in this area (either alone or in combination with other active substances) is limited. The present study will address this. It is anticipated that the use of NIRS to assess haemodynamics during cognitive performance, in conjunction with EEG will provide a more thorough insight into the effects of these extracts, particularly in light of the disparity in findings to date in relation to cerebro-electrical activity.

The tasks chosen in the current study are those that have previously been shown to be sensitive to one or both of the extracts, for example, serial 3s subtractions, serial 7s subtractions and RVIP (Kennedy et al., 2004; Kennedy et al., 2002; Reay et al., 2005, 2006). Tasks were also selected based on their ability to activate the pre-frontal cortex and in this regard, the 3-back task and Stroop task were also included in addition to serial 7s subtractions and RVIP (Cohen et al., 1997; Drummond et al., 1999; Lawrence, Ross, & Stein, 2002; Schroeter et al., 2002).

The majority of ginseng studies have looked at doses of either 200 mg and 400 mg with positive effects demonstrated at both doses (Dangelo et al., 1986; Kennedy, Reay, & Scholey, 2007; Kennedy et al., 2004; Kennedy et al., 2001b, 2002; Reay et al., 2005; Reay et al., 2010; Sorensen & Sonne, 1996; Sunram-Lea et al., 2005), whereas ginkgo studies have focussed on doses of 120 mg, 180 mg, 240 mg and 360 mg, with an absence of research into the effects of a ~200 mg dose. An increment of this proportion may appear small; however, when the differences in behavioural and physiological effects at the aforementioned doses are considered, it could be that even a slight variation in ginkgo dose may be of significance. Therefore, with previous EEG research demonstrating significant effects of ginseng following a 200 mg dose and with limited research into the effects of this dose overall for ginkgo, it seemed appropriate to

investigate the effects of these extracts at ~200 mg. Incorporating both a chronic and an acute assessment extends the research in healthy, young populations, delineating whether the effects are cumulative or have more impact following a single dose. The time course of acute assessment (beginning at 2 hours post-dose and continuing for approximately 1 hour) was chosen based on previous acute behavioural research. In young healthy adults, an effect of these extracts either alone and/or in combination has been identified within this time-frame (Kennedy et al., 2004; Kennedy et al., 2000, 2001a, 2002).

The aim of the present study was to assess the effects of two herbal extracts known to have psychoactive properties, in order to explore their effects on cerebro-electrical activity and the haemodynamic response to behavioural task performance. This randomised double-blind, placebo-controlled, balanced, crossover study therefore examined the acute and chronic effects of *Ginkgo biloba* and a *Ginkgo biloba*/*Panax ginseng* combination, on brain activity as assessed by EEG and NIRS, during the completion of a range of cognitive tasks administered by the Computerised Mental Performance Assessment System (COMPASS).

2.2 Method

2.2.1 Participants

Eighteen healthy young volunteers (6 males, 12 females) between the ages of 19 and 38 (mean age 22.9, SD 5.04; BMI 23.8, SD 2.7) were recruited. The study was approved by Northumbria University's School of Psychology and Sports Sciences ethics committee and was conducted in accordance with the Declaration of Helsinki. Prior to participation in the study, participants were required to provide informed consent. All participants reported that they were in good health and were taking no illicit drugs. Additionally, they were free from any 'over the counter', herbal or prescribed medications, with the exception of the contraceptive pill for some female participants. They had not taken any dietary supplements in the past 3 months and were not pregnant or seeking to

become pregnant. Habitual smokers consuming more than 3 cigarettes per day were excluded from the study as were those who consumed more than 6 cups of coffee per day (or the equivalent in caffeine from other sources).

2.2.2 Design and treatment

A randomised, double-blind, counter-balanced, within subjects, placebo-controlled design was utilised. Participants attended six study visits and at visits 1, 3, and 5 were randomised to receive 1 of the following treatments; 207 mg *Ginkgo biloba*; 207 mg *Ginkgo biloba* and 207 mg *Panax ginseng* in combination, or placebo. Each treatment was administered in the form of 6 tablets containing either standardised dried extract of *Ginkgo Biloba* leaves (DER (drug to extract ratio) 50:1) or a combination of dried extract of *Ginkgo Biloba* leaves (DER 50:1) and standardised dried extract of *Panax Ginseng* root (DER 3, 6:1), or placebo (all treatments were supplied by Phytolab GmbH & Co., Frankfurt). The participant remained blind to the treatment they had received. Participants were randomly allocated to a treatment regime using a Latin square design that counterbalanced the order of treatments across the cohort and treatment periods. Each treatment was ingested for a total of 15 days, with a 13-day no-treatment wash-out between each. All participants met the 80 % treatment compliance level across the study period.

2.2.3 Physiological, cognitive and mood measures

2.2.3.1 Near-infrared spectroscopy measurements

Relative changes in the absorption of near infrared light were measured at a time resolution of 10 Hz using a 12 channel Oxymon system (Artinis Medical Systems B.V.). The system emitted 2 nominal wavelengths of light (~765 and 855 nm) with an emitter/optode separation distance of 4 cm. The differential pathlength factor was adjusted according to the age of the participant. Relative concentration changes in oxy-Hb, deoxy-Hb and total-Hb were calculated by means of a modified Beer–Lambert law using the proprietorial software. In this study, a simple two emitter/receiver optode pair

configuration was utilised (i.e. 2 channels) (see figure 1.1). The emitter/receiver optode pairs were positioned over the left and right pre-frontal cortex using a standard optode holder headband (each emitter optode was at a 16.5 mm distance from the midline and 4 cm laterally from the corresponding receiver optode). This separated the emitter/receiver pairs from each other by 33 mm.

2.2.3.2 Cognitive and mood measures

Cognitive measures were delivered using the Computerised Mental Performance Assessment System (COMPASS), University of Northumbria at Newcastle, a purpose designed software application for the flexible delivery of randomly generated parallel versions of standard and novel cognitive assessment tasks that has previously been shown to be sensitive to nutritional interventions e.g. (Haskell et al., 2010; Kennedy, Veasey, et al., 2010). The tasks were chosen based on their ability to activate the pre-frontal cortex (Cohen et al., 1997; Drummond et al., 1999; Lawrence et al., 2002; Schroeter et al., 2002) and/or their known sensitivity to one or both of the nutritional interventions under investigation (Kennedy et al., 2004; Kennedy et al., 2002; Reay et al., 2005). The tasks were delivered in the following order; serial 3s subtraction task (2 minutes), serial 7s subtraction task (2 minutes), a rapid visual information processing task (RVIP – 5 mins), mental fatigue visual analogue scale, Stroop task (4 minutes) and the N-back (3-back task) (2 minutes).

2.2.3.2.1 Serial 3s:

The serial subtractions task is a measure of concentration and working memory, which can be manipulated in terms of its cognitive load, by augmenting the number to be subtracted. Since it was first described, as a tool for the measurement of mental impairment (Hayman, 1942), it has been used in a number of studies assessing cognitive ability in the presence of a range of nutritional interventions e.g. (Kennedy, Wightman, et al., 2010; Reay et al., 2006; Scholey et al., 2001). In this task, a starting number between 800 and 999 appears on the screen and participants are instructed to count backwards as quickly and as accurately as possible from this number in 3s, using the keyboards linear number keys to make their response. Each digit is represented on screen by an asterisk

and responses are cleared when the 'enter' key is pressed. Participants are only shown one number on screen and the rest of the numbers are generated by subtracting from the previous number in their head. In the case of incorrect responses, subsequent responses are scored as positive if they are correct in relation to the new number. This task was scored for number of correct responses and number of errors.

2.2.3.2.2 Serial 7s:

The serial 7s task was identical to the serial 3s task, except that it involved serial subtraction of 7s.

2.2.3.2.3 Rapid visual information processing task (RVIP):

The RVIP task is a measure of sustained attention and working memory that is known to give rise to fronto-parietal activation (Coull, Frith, Frackowiak, & Grasby, 1996). During this task, the participant monitors a continuous series of digits for targets of 3 consecutive odd or 3 consecutive even digits. The digits are presented on the computer screen at the rate of 100 per minute in pseudo-random order and the participant responds to the detection of a target string by pressing the space bar as quickly as possible. The task is continuous and lasts for 5 minutes, with 8 correct target strings being presented in each minute. This task was scored for percentage of target strings correctly detected, average reaction time for correct detections, and number of false alarms.

2.2.3.2.4 Stroop:

The Stroop task is a response inhibition task and a measure of executive functioning and is known to activate the pre-frontal cortex (Cabeza & Nyberg, 2000). In this task, a series of colour names appear on the screen one at a time in different coloured fonts. Participants are required to use a colour coded response pad to select the colour that matches the colour font that the word is written in. The words that are presented are either 'congruent' (name of colour and colour of ink the same) or 'incongruent' (name of colour and colour of ink different) and are presented randomly. Participants are asked to respond as quickly and as accurately as possible. The task lasts for 4 minutes and the number of stimuli presented are determined by the speed at

which the participant responds. This task was scored for percentage accuracy and reaction time.

2.2.3.2.5 3-Back:

N-back is a task that assesses working memory that is known to activate the pre-frontal cortex (Owen, McMillan, Laird, & Bullmore, 2005). In the present study, a 3-back version of the task was utilised. A series of letters appear on the screen one at a time and participants have to identify whether that letter was presented 3 letters previously in the series of letters or not by pressing corresponding 'yes' and 'no' buttons using keys on the keyboard. Participants were required to respond to 45 letter presentations, with 15 target letters. This task was scored for percentage of correct responses and reaction time.

2.2.3.2.6 Subjective mental fatigue visual analogue scale:

Participants were asked to rate how mentally fatigued they felt by placing an 'x' on a 100 mm line with the end points labelled 'not at all' (left hand end) and 'extremely' (right hand end).

2.2.3.3 Electroencephalography measurements

EEG was recorded from 32+2 channels using an electrode cap (sintered silver-silver chloride electrodes; BioSemi B.V. The Netherlands) according to the international 10-20 system. EEG was obtained using BioSemi ActiveTwo amplifier (BioSemi, B.V.) and Acquire 4.3 software (Neuroscan). Amp settings during the recording periods were as follows: low pass 30 Hz, high pass 0.46 Hz. The montage included four midline sites (FZ; CZ; PZ; OZ), 14 sites over the left hemisphere (FP1; AF3; F3; F7; FC1; FC5; C3; T7; CP1; CP5; P3; P7; PO3; O1) and 14 sites over the right hemisphere (FP2; AF4; F4; F8; FC2; FC6; C4; T8; CP2; CP6; P4; P8; PO4; O2), all referred to linked mastoids (see figure 2.1). Inter-electrode impedance levels were kept below 5 kΩ. Participants were asked to visually fixate on a small cross, initially displayed on the monitor, to minimise eye movements during all eyes open EEG recordings. Any sections of the EEG recording still contaminated with eye movements, muscular activity, or other artefacts were excluded from the analysis using automatic eye-blink correction, artefact rejection carried out using Edit 4.3 (Neuroscan). Event-related potentials and waveband analyses were conducted

using Edit 4.3 (Neuroscan). Time windows of 300-500 milliseconds (ms), 280-700 ms and 180-280 ms were used to respectively capture P3a, P3b and P2 ERP components.

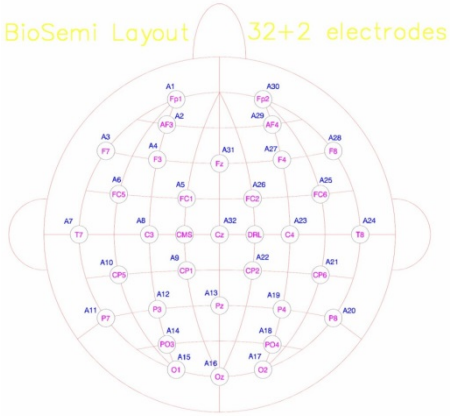


Figure 2.1 Example of EEG electrode set-up as used in current chapter. Taken from BioSemi website; http://www.biosemi.com/index.htm (BioSemi, 2016).

2.2.3.3.1 Three-stimulus odd ball task:

The task was administered in three, 4-minute blocks. Participants were instructed to press the spacebar, every time they identified a target stimulus (small green square) and to ignore all other stimuli (frequent stimuli: small red circle and novel stimuli: large blue square). The experimental tasks presented 450 stimuli in total, which comprised the following; 60 target stimuli, 330 frequent stimuli and 60 novel stimuli. The stimuli remained on the screen for 100 ms. Immediately pre and post task, 40 seconds of continuous resting eyes open and eyes closed activity was recorded. The order of which was randomly generated by the computer. Prior to the experimental blocks at each session, a practice block was administered without the novel stimuli.

2.2.4 Procedure

Participants attended the Brain, Performance and Nutrition Research Centre (BPNRC) at Northumbria University on 7 separate occasions (introductory (screening) visit and Study Visits 1, 2, 3, 4, 5, 6) with each of the 6 study visits separated by 2 weeks. Testing took place in a suite of testing facilities and participants were assessed individually. The screening visit comprised: informing volunteers about the nature of the

study, its requirements and restrictions and obtaining informed consent. Demographic data was then collected and familiarisation with the testing battery was completed. Once participants' eligibility to participate was confirmed, they were randomly allocated, utilising a computer generated random number list, to a position on a Latin Square, which counterbalanced the order of treatments across the cohort and study days. The remaining 6 study visits were identical apart from the treatment administered. Each visit involved attending the laboratory between 8:30 am and 9.30 am on 6 separate occasions, 2 weeks apart (visits 1, 2, 3, 4, 5, 6). Participants were required to fast since waking and abstain from alcohol from 8.30 pm the previous evening. On each of the 6 visits, participants were assessed with regards compliance to the study requirements and restrictions and were then asked to take the appropriate day's investigational product. They were then provided with a standardised light breakfast of 2 slices of buttered toast with jam and a 200 ml glass of orange juice. Two hours after treatment consumption, they underwent assessment of cerebro-electrical activity at rest (40 seconds eyes open and 40 seconds eyes closed) and during the 3-stimulus odd ball task utilising EEG. Immediately following this, they underwent a NIRS assessment of cerebral oxygenation parameters at rest (a 4-minute period where participants were required to clear their mind and concentrate on their breathing) and during tasks delivered by the COMPASS testing battery relevant to frontal areas of the brain. After testing for the day had ceased, participants were given 13 days' supply of the treatment to take home with them, to take between visits 1-2, 3-4 and 5-6. During visits 2-3 and 4-5 a no-treatment 'wash out' period was observed. Upon return to the lab at visits 2, 4 and 6, participants were administered day 15 of the relevant treatment in the lab. Consequently, at visits 1, 3 and 5 participants consumed day 1 of their treatment and completed their 'acute' assessment. At visits 2, 4 and 6 participants consumed day 15 of their treatment and completed their 'chronic' assessment in the presence of an acute dose. The study outline is shown in fig. 2.2 and the testing schedule for each visit is shown in fig.2.3

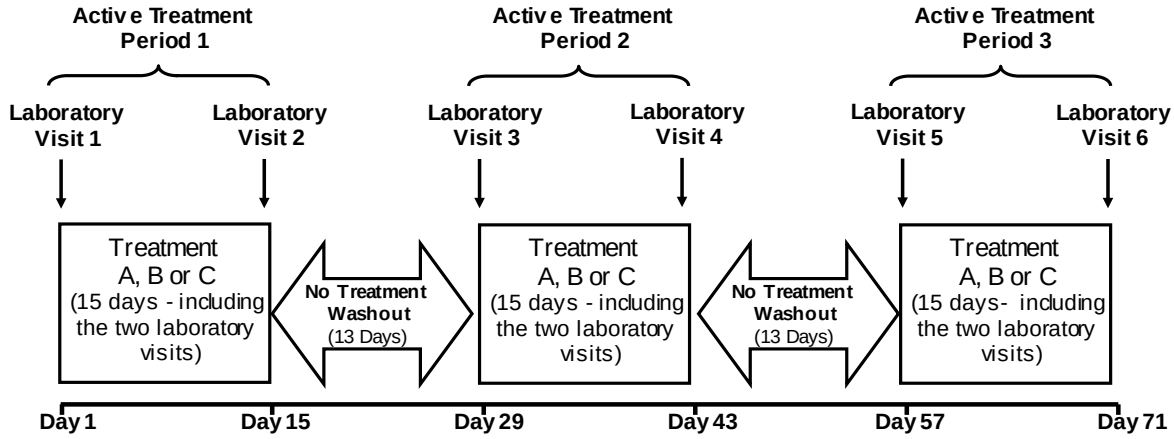


Fig. 2.2. Treatment and study outline across the 10 weeks of the study. Individual participants took each of the 3 treatments (ginkgo, ginkgo/ginseng, placebo) during one of the three 15-day active treatment periods in a counterbalanced order (to eliminate order effects). Cognitive function/electrical activity and haemodynamic response to task performance was tested on the first and last day of this period. A 13 day no treatment wash-out was interposed between active treatment periods.

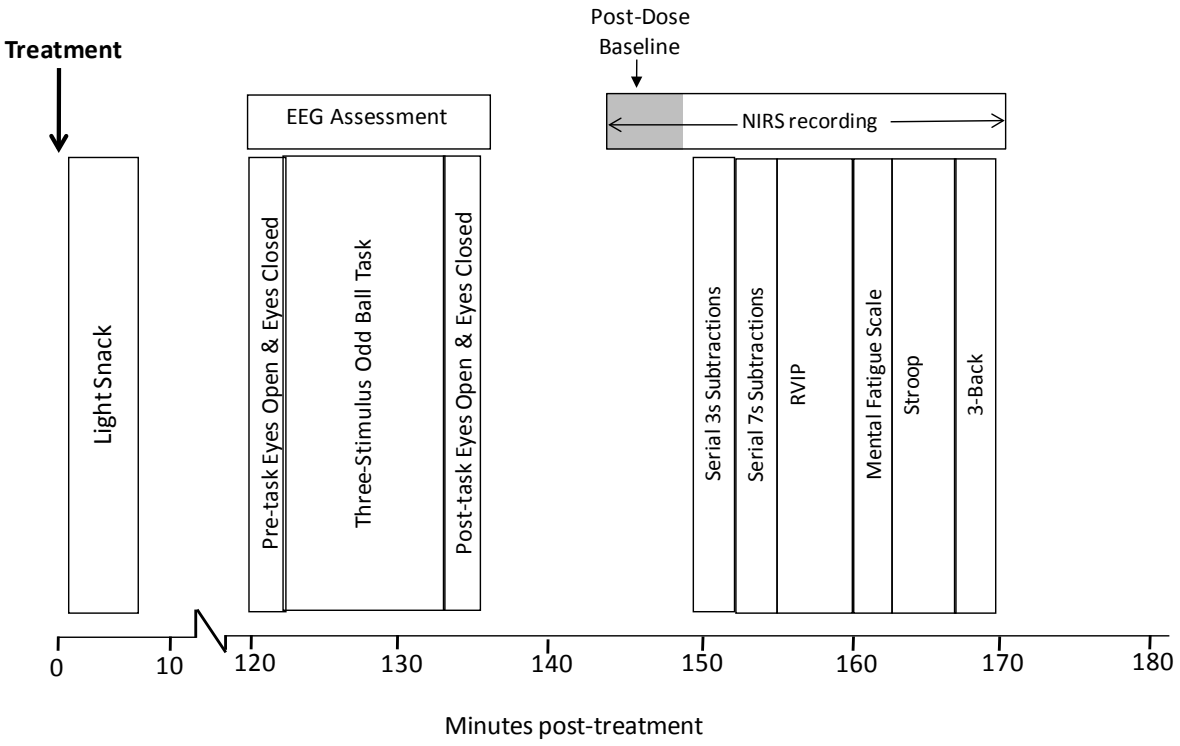


Fig. 2.3. Testing schedule for Visits 1, 2, 3, 4, 5 and 6

2.2.5 Statistics

Prior to the primary NIRS analysis a within subjects ANOVA was carried out with left/right optode included as a factor to examine any treatment related hemispheric

differences in response. As there were no interpretable interactions involving this factor the data from the 2 channels were averaged for the analysis.

For the primary NIRS analysis, the question under investigation was whether *Ginkgo biloba*, or *Ginkgo biloba* and *Panax ginseng* in combination, would modulate the haemodynamic response to overall task performance in comparison to placebo and if acute/chronic dosing was a factor in this response. Data for oxy-Hb, deoxy-Hb and total-Hb was averaged across the duration of each individual task (2, 4 or 5 minutes) baseline adjusted to the 4 minute, post-dose, pre-task resting baseline measurement. It was then analysed by 3-way repeated measures ANOVA (treatment (207 mg *Ginkgo biloba*, 207 mg *Ginkgo biloba* and 207 mg *Panax ginseng* in combination, or placebo) X task (serial 3s, serial 7s, RVIP, Stroop, 3-back) X day (acute and chronic)). Significant treatment related interactions were then described with reference to *a priori* planned comparisons, in which averaged data from each task epoch for each active treatment was compared to placebo utilising t-tests calculated with the Mean Squares Error from the ANOVA (Keppel, 1991). In order to reduce the potential for Type I errors only those planned comparisons associated with a statistically significant difference on the initial ANOVA are reported. In addition, only those instances where a consistent pattern of significant differences are maintained across epochs are identified as reportable significant effects.

In order to identify if there was a pattern of effects within each task over time and if the haemodynamic response was modulated differently throughout the course of task performance, data from each task was broken down into smaller, 10 second epochs and analysed together as a series of epochs over time. Secondary analysis of the NIRS data therefore involved averaging each task across 10 second epochs and baseline adjusting to the 4-minute pre-task resting baseline measurement. It was then analysed by 3-way repeated measures ANOVA (treatment (as per primary analysis) X 10 second epoch (90) X day (as per primary analysis). Planned comparisons were conducted as per primary analysis, documented above.

Following transformation of the EEG data, the effects of the treatment on the latency and amplitude of evoked potentials were assessed by 3-way ANOVA (treatment (as above) X scalp site (P3a, P3b, P2) X day (as above)). Electrode scalp sites were as follows; sites FZ and CZ (P3a), PZ and CZ (P3b) and FZ and CZ (P2). Delta, theta, alpha and beta waveband activity is represented as statistical maps.

Cognitive performance and subjective mood were analysed by 2-way repeated measures ANOVA (treatment (as above) X day (as above). Significant treatment related effects were described with reference to *a priori* planned comparisons (as above) where each active treatment was compared to placebo.

Performance on the oddball task was analysed by repeated measures, two-way ANOVA (treatment (as above) X day (as above).

2.3 Results

2.3.1 Near infrared spectroscopy

2.3.1.1 Primary analysis

Effects of treatment on cerebral haemodynamics over time during task performance.

2.3.1.1.1 Oxygenated haemoglobin

There were no treatment-related differences on oxy-Hb, see fig. 2.4a.

2.3.1.1.2 Deoxygenated haemoglobin

A significant interaction effect (treatment X task) was observed for deoxy-Hb [$F(8, 136)=2.50$, $p=0.014$]. Planned comparisons revealed that irrespective of acute or chronic dosing, deoxy-Hb was significantly increased during the Stroop [$t(136)=3.44$, $p<0.0008$, $d=-0.53$] and 3-back tasks [$t(136)=3.28$, $p=0.0013$, $d=-0.49$] following ginkgo as compared to placebo, see fig. 2.4b.

2.3.1.1.3 Total haemoglobin

There were no treatment-related differences on total-Hb, see fig. 2.4c.

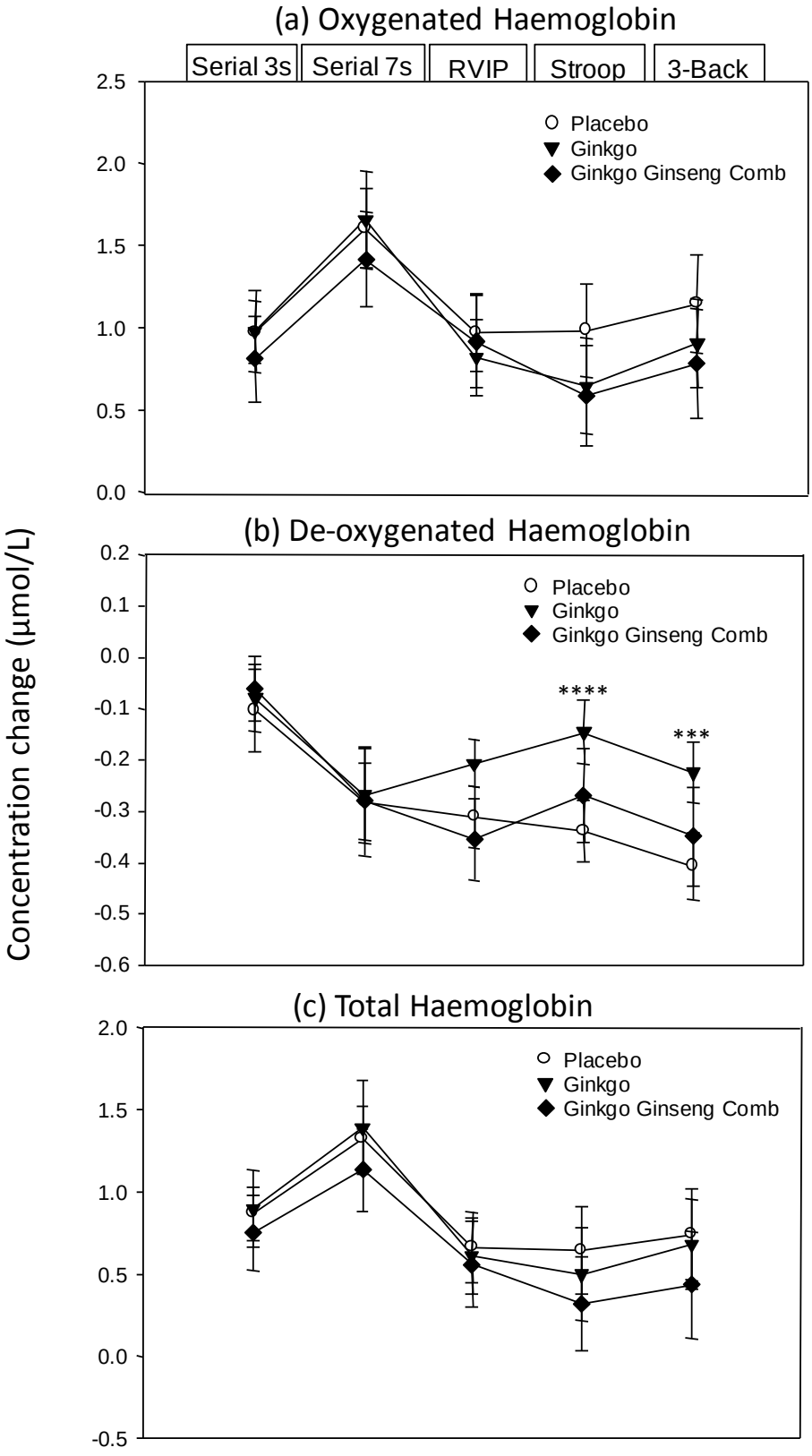


Fig. 2.4. Concentration changes of oxy-Hb (a), deoxy-Hb (b) and total-Hb (c) during cognitive tasks following placebo, 207 mg *G. biloba*, or 207 mg *G. biloba* and *P. ginseng*. Means and SEM are presented. Treatment X task interaction effects are shown for (b) deoxy-Hb. Significance is compared to placebo (t-tests calculated with the Mean Squares Error from the ANOVA) (*** $p<0.005$, **** $p<0.001$).

2.3.1.2 Secondary analysis

Effects of treatment on cerebral haemodynamics during task performance using smaller duration (10 second) epochs.

2.3.1.2.1 Oxygenated haemoglobin

There were no treatment-related differences on oxy-Hb, see fig. 2.5a.

2.3.1.2.2 Deoxygenated haemoglobin

A significant interaction effect (treatment X epoch) was observed for deoxy-Hb [$F(178, 3026)=1.90$, $p<0.001$]. Planned comparisons revealed that irrespective of acute or chronic dosing, deoxy-Hb was significantly increased consistently throughout the Stroop task and 3-back tasks following ginkgo as compared to placebo and at individual epochs during RVIP, see fig. 2.5b for details of significant effects at each epoch.

2.3.1.2.3 Total haemoglobin

There were no treatment-related differences on total-Hb, see fig. 2.6.

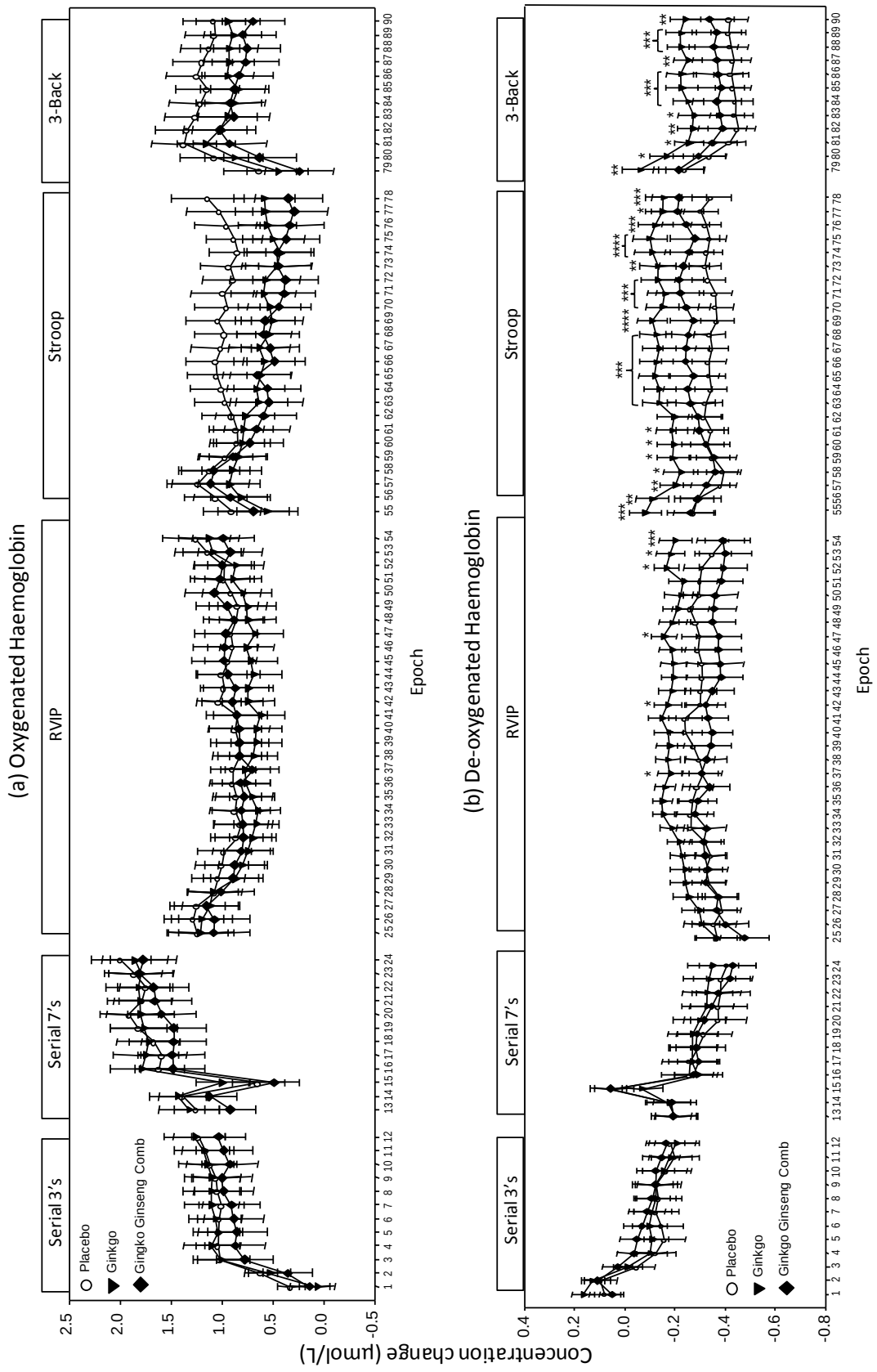


Fig. 2.5. Concentration changes of oxy-Hb (a) and deoxy-Hb (b) during cognitive tasks following placebo, 207 mg *G. biloba*, or 207 mg *G. biloba* and *P. ginseng*. Means and SEM are presented. Treatment X task interaction effects are shown for (b) deoxy-Hb. Significance is compared to placebo (t-tests calculated with the Mean Squares Error from the ANOVA) (* $p<0.05$, ** $p<0.01$, *** $p<0.005$, **** $p<0.001$).

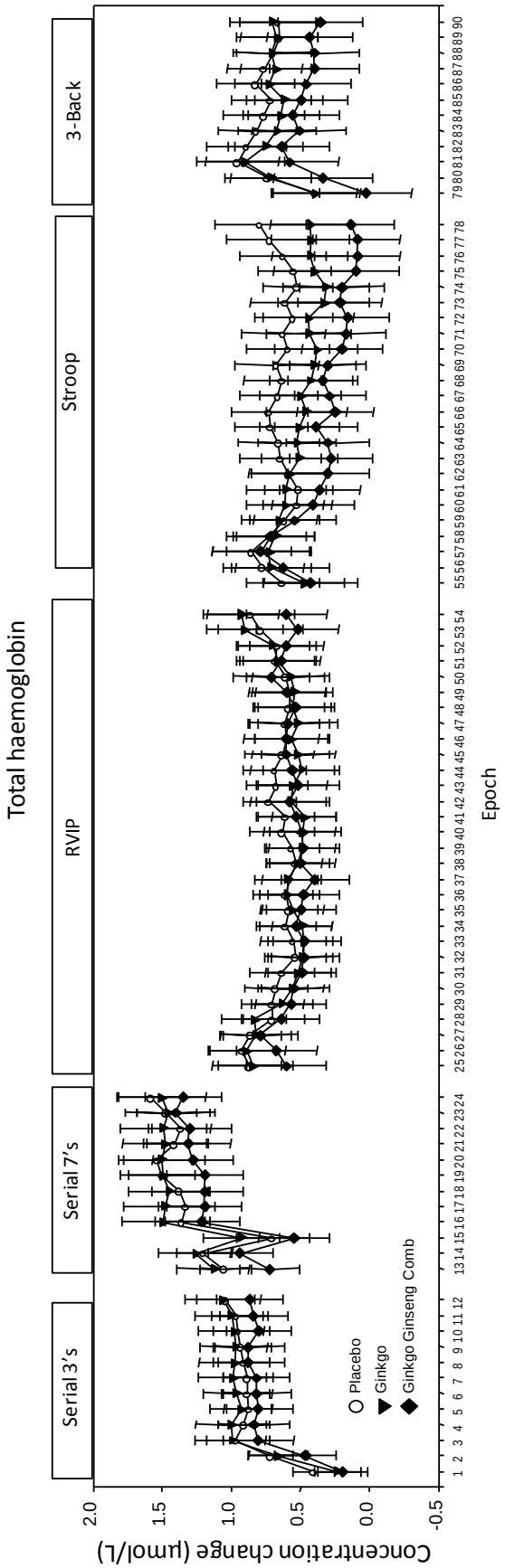


Fig. 2.6. Concentration changes of total-Hb during cognitive tasks following placebo, 207 mg *G. biloba*, or 207 mg *G. biloba* and *P. ginseng*. Means and SEM are presented.

2.3.2 Cognitive performance and mood

2.3.2.1 Serial 7s

There was a significant main effect of treatment on serial 7s subtractions errors [$F(2, 32)=4.05$, $p=0.027$]. However, planned comparisons revealed that there were no significant differences on this measure between active treatments and placebo.

2.3.2.2 RVIP

There was a significant treatment x day interaction for number of RVIP false alarms [$F(2, 34)=4.22$, $p=0.023$]. Planned comparisons revealed that following the acute dose of ginkgo/ginseng combination, there was a significant increase in the number of false alarms as compared to placebo [t (34)=2.22, $p=0.033$, $d=-0.62$], see fig. 2.7.

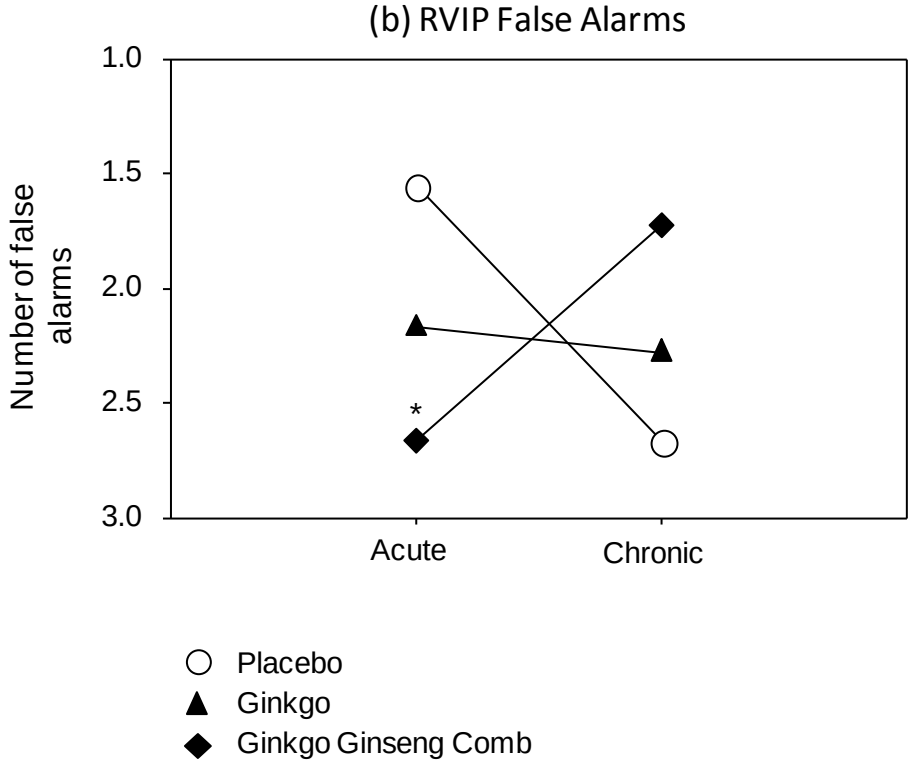


Fig. 2.7. Number of false alarms on RVIP task following ginkgo, ginkgo/ginseng combination and placebo following acute and chronic treatment (* p<0.05).

Table 2.1. Raw scores for serial subtractions, RVIP, mental fatigue, Stroop and 3-back tasks for each treatment. Means ±SEM values are presented with F and p values from the primary ANOVA of treatment effects and treatment x acute/chronic dose interactions. Significant measures are shown in bold.

Measure	*n*	Treatment	120 minutes post-dose		Treat effect	Treat x day interaction
			Acute	Chronic		
Serial 3s subs correct (number)	18	Placebo	40.83±3.25	42.94±3.11	F<1	F=1.13 p>0.1
		Ginkgo	41.56±3.02	41.22±2.70		
		Ginkgo/Ginseng	40.89±3.04	39.94±2.80		
Serial 3s subs errors (number)	18	Placebo	1.94±0.48	1.78±0.44	F<1	F=1.07 p>0.1
		Ginkgo	2.00±0.30	2.44±0.54		
		Ginkgo/Ginseng	1.67±0.38	2.94±0.56		
Serial 7s subs correct (number)	17	Placebo	26.24±2.09	25.47±2.23	F<1	F=1.01 p>0.1
		Ginkgo	24.71±2.62	26.12±2.23		
		Ginkgo/Ginseng	25.59±2.53	25.76±2.35		
Serial 7s subs errors (number)	17	Placebo	1.82±0.44	2.18±0.37	**F=4.05 p=0.03**	F<1
		Ginkgo	3.24±0.74	2.94±0.75		
		Ginkgo/Ginseng	2.29±0.64	2.53±0.42		
RVIP correct (%)	18	Placebo	74.17±4.10	68.75±3.75	F<1	F=2.57 p=0.09
		Ginkgo	72.22±4.51	69.58±4.31		
		Ginkgo/Ginseng	70.69±3.56	73.75±3.61		
RVIP RT (ms)	18	Placebo	464.74±14.63	472.48±15.70	F=1.13 p>0.1	F=1.67 p>0.1
		Ginkgo	463.11±18.68	445.55±16.91		
		Ginkgo/Ginseng	463.75±14.14	457.61±12.67		
RVIP false alarms (number)	18	Placebo	1.56±0.44	2.67±0.66	F<1	**F=4.22 p=0.02**
		Ginkgo	2.17±0.47	2.28±0.55		
		Ginkgo/Ginseng	**2.67±0.40**	1.72±0.36		
Mental fatigue (mm)	18	Placebo	53.67±4.39	57.61±4.40	F<1	F<1
		Ginkgo	55.17±5.34	56.33±4.76		
		Ginkgo/Ginseng	53.67±4.27	52.94±4.56		
Stroop overall correct (%)	18	Placebo	98.21±0.46	98.18±0.38	F<1	F<1
		Ginkgo	98.15±0.44	98.11±0.35		
		Ginkgo/Ginseng	98.40±0.34	98.39±0.35		
Stroop overall RT (ms)	18	Placebo	656.28±28.43	648.11±23.51	F<1	F<1
		Ginkgo	655±21.49	654.89±22.94		
		Ginkgo/Ginseng	647.28±21.48	649.78±21.64		
3-back correct (%)	18	Placebo	92.59±1.51	91.11±1.69	F<1	F<1
		Ginkgo	91.85±1.76	87.65±3.60		
		Ginkgo/Ginseng	89.01±3.65	90±3.61		
3-back RT (ms)	18	Placebo	1283±161	1326±171	F<1	F<1
		Ginkgo	1313±183	1229±173		
		Ginkgo/Ginseng	1246±151	1262±167		

2.3.3 Electroencephalography measurements

2.3.3.1 Waveband activity

The mean power (post-task eyes closed) for the frequency bands (delta, theta, alpha and beta) in the individual brain regions following ingestion of either 207 mg *G. biloba*, or 207 mg *G. biloba* and 207mg *P. ginseng* as compared to placebo are presented in table 2.2.

		Acute (Day 1) Mean power (μV^2/Hz) 'eyes closed'			Chronic (Day 15) Mean power (μV^2/Hz) 'eyes closed'		
Waveband	**Scalp region**	**Placebo**	**Ginkgo**	**Ginkgo/Ginseng combination**	**Placebo**	**Ginkgo**	**Ginkgo/Ginseng combination**
Delta	Frontal	27.95	50.39	34.53	30.01	38.72	42.86
(0.50-4.00 Hz)	Left frontal	21.50	22.91	23.99	25.10	23.48	23.77
	Right frontal	20.64	51.56	26.71	28.73	29.11	24.60
	Left parietal	8.50	10.04	10.31	9.24	9.49	10.67
	Right parietal	9.83	10.17	10.55	9.65	10.64	11.70
	Occipital	13.99	14.08	15.01	13.27	12.57	15.27
Theta	Frontal	1.90	2.52	2.86	2.34	2.30	2.25
(4.00-8.00Hz)	Left frontal	1.83	2.35	2.59	2.11	2.02	2.14
	Right frontal	1.73	2.39	2.23	1.98	2.04	2.13
	Left parietal	1.90	2.40	2.51	2.04	2.16	2.11
	Right parietal	2.05	2.71	2.69	2.27	2.11	2.02
	Occipital	3.55	4.35	4.11	3.60	3.80	3.51
Alpha	Frontal	5.91	6.55	5.81	4.43	5.77	5.01
(8.00-11.00Hz,	Left frontal	4.69	5.08	4.69	3.96	4.53	4.24
11.00-14.0Hz)	Right frontal	4.84	5.58	4.61	3.89	4.93	4.37
	Left parietal	7.91	10.29	8.48	7.39	8.85	7.59
	Right parietal	8.06	9.19	7.93	7.24	8.71	7.13
	Occipital	23.49	25.83	21.50	18.88	23.71	22.22
Beta	Frontal	0.47	0.44	0.43	0.40	0.45	0.47
(14.00-25.00Hz,	Left frontal	0.37	0.43	0.38	0.34	0.37	0.37
25.00-35.00Hz)	Right frontal	0.38	0.42	0.38	0.33	0.37	0.38
	Left parietal	0.42	0.53	0.48	0.42	0.46	0.45
	Right parietal	0.44	0.54	0.48	0.42	0.46	0.48
	Occipital	0.94	1.20	1.02	0.87	1.03	0.99

Table 2.2. Mean 'eyes closed' power in the delta, theta, alpha and beta wavebands for ginkgo, ginkgo/ginseng combination, and placebo.

2.3.3.1.1 Acute effects (post-task eyes closed)

Demonstration of the effects of acute ingestion of ginkgo and ginkgo/ginseng combination as compared to placebo are presented as descriptive probability maps. Associated significant areas are highlighted. See figures 2.8 and 2.9.

2.3.3.1.1.1 Delta (0.50-4.00 Hz)

Both ginkgo and ginkgo/ginseng combination led to an increase in frontal delta activity, with the combination treatment also leading to an increase in left and right parietal activity, as compared to placebo.

2.3.3.1.1.2 Theta (4.00-8.00 Hz)

The ginkgo/ginseng combination led to an increase in theta activity in the frontal and right parietal regions as compared to placebo.

2.3.3.1.1.3 Alpha (8.00-11.00 Hz, 11.00-14.00 Hz)

Neither ginkgo or the ginkgo/ginseng combination led to a change in alpha activity as compared to placebo.

2.3.3.1.1.4 Beta (14.00-25.00 Hz, 25.00-35.00 Hz)

Both ginkgo and ginkgo/ginseng combination led to a reduction in frontal beta activity and ginkgo led to an increase in right parietal beta activity as compared to placebo.

2.3.3.1.2 Chronic effects (Post-Task Eyes Closed)

Demonstration of the effects of chronic ingestion of ginkgo and ginkgo/ginseng combination as compared to placebo are presented as descriptive probability maps. Associated significant areas are highlighted. See figures 2.10 and 2.11.

2.3.3.1.2.1 Delta (0.50-4.00 Hz)

Ginkgo led to an increase in frontal and left occipital delta activity and the ginkgo/ginseng combination led to an increase in frontal delta activity as compared to placebo. The increase in frontal activity was more marked for the combination treatment, than for ginkgo alone.

2.3.3.1.2.2 Theta (4.00-8.00 Hz)

Neither ginkgo or ginkgo/ginseng combination led to a change in theta activity as compared to placebo.

2.3.3.1.2.3 Alpha (8.00-11.00 Hz, 11.00-14.00 Hz)

Neither ginkgo or ginkgo/ginseng combination led to a change in alpha activity as compared placebo.

2.3.3.1.2.4 Beta (14.00-25.00 Hz, 25.00-35.00 Hz)

The ginkgo/ginseng combination led to an increase in right parietal beta activity in the 25.00-35.00Hz range only, as compared to placebo.

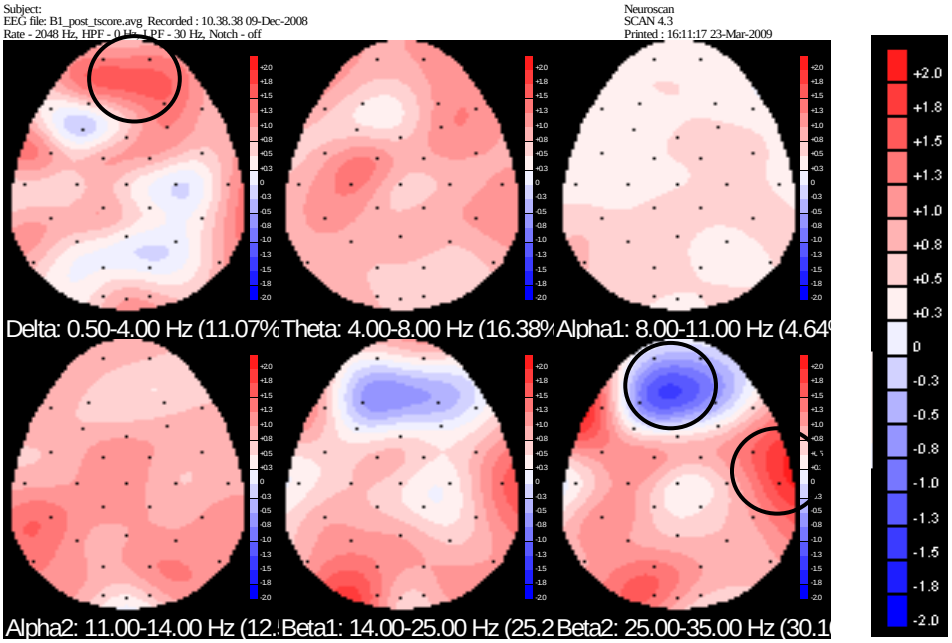


Fig. 2.8. Descriptive probability maps showing increases in EEG power in frontal delta and right parietal beta and a reduction in frontal beta wavebands in comparison to placebo following acute dose of ginkgo.

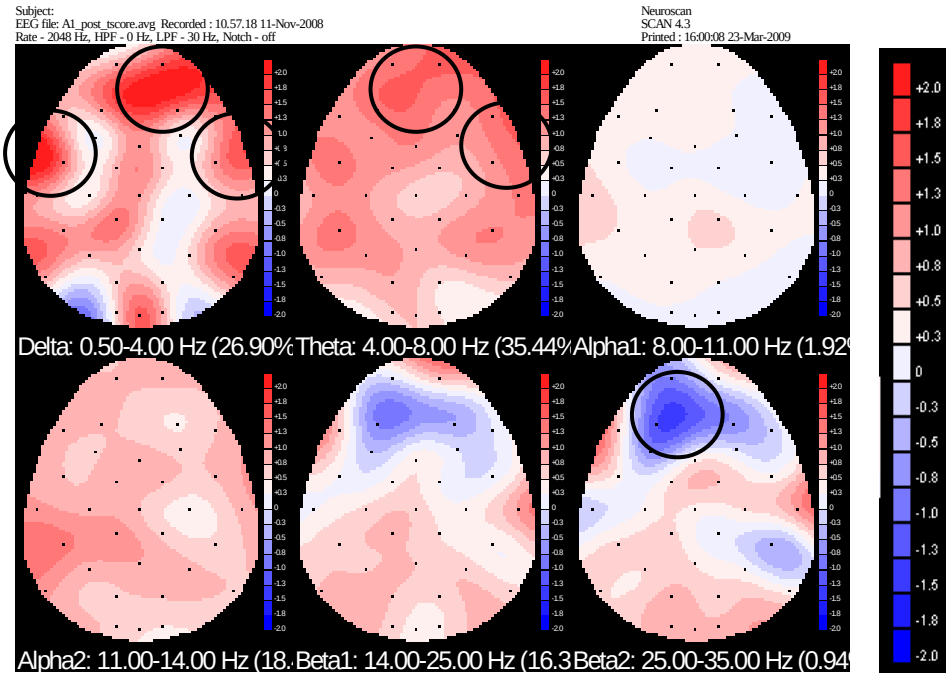


Fig. 2.9. Descriptive probability maps showing increases in EEG power in frontal and parietal delta and theta wavebands and a reduction in frontal beta wavebands in comparison to placebo following acute dose of ginkgo/ginseng combination.

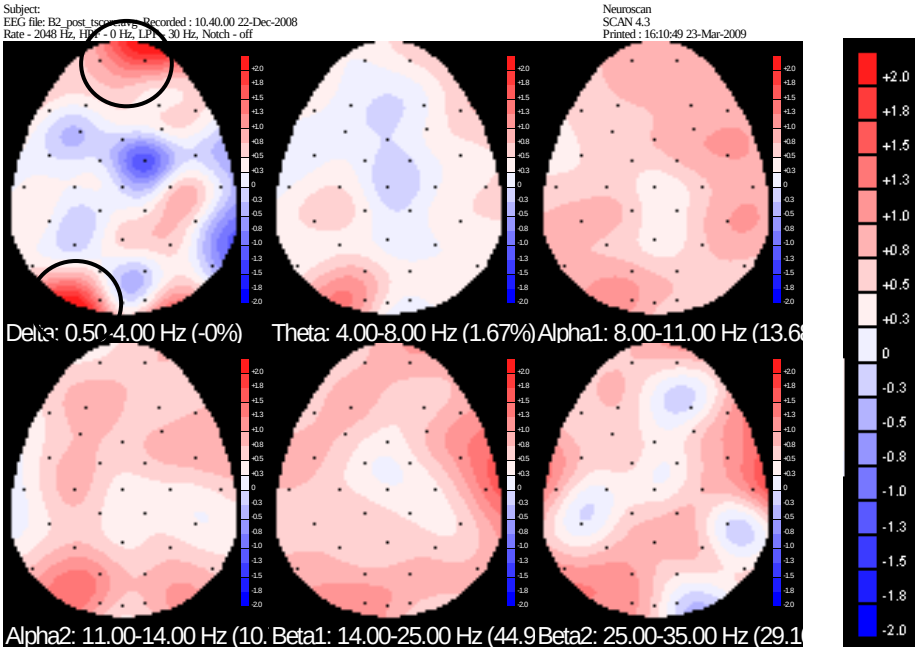


Fig. 2.10. Descriptive probability maps showing increases in EEG power in frontal and left occipital delta wavebands in comparison to placebo following chronic dose of ginkgo.

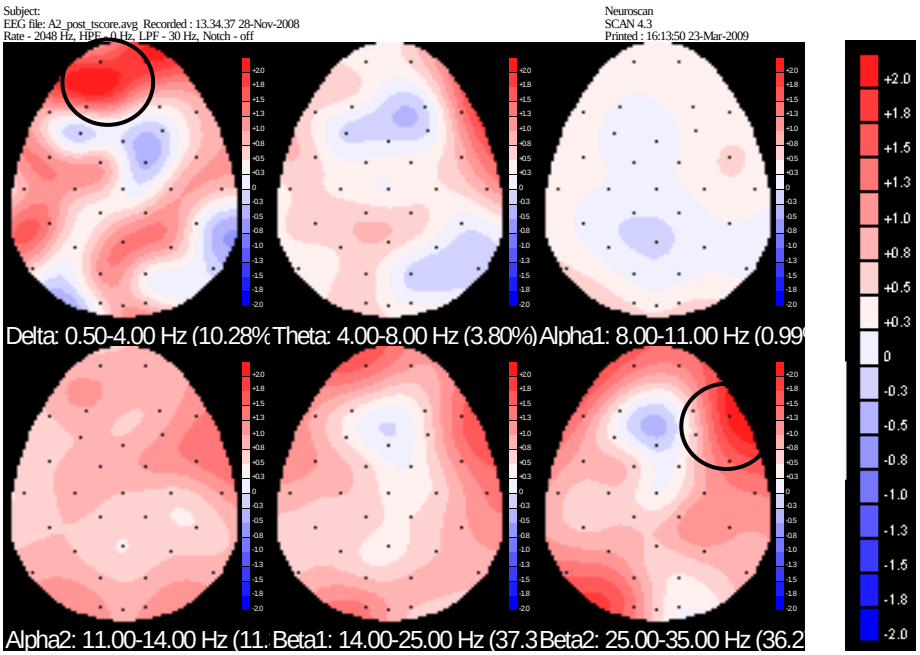


Fig. 2.11. Descriptive probability maps showing increases in EEG power in frontal delta and right parietal beta wavebands in comparison to placebo following chronic dose of ginkgo/ginseng combination.

2.3.3.2 P300 Latency and amplitude

2.3.3.2.1 Amplitude

There were no significant differences between placebo and each of the active treatments following either acute or chronic doses in amplitude of P3a, P3b or P2 ERP components.

2.3.3.2.2 Latency

There were no significant differences between placebo and each of the active treatments following either acute or chronic doses in latency of P3a, P3b or P2 ERP components.

2.3.3.3 EEG task performance

2.3.3.3.1 Odd ball task accuracy

There were no significant differences between placebo and each of the active treatments following either the acute or chronic doses.

2.3.3.3.2 Odd ball task reaction time

There were no significant differences between placebo and each of the active treatments following either the acute or chronic doses.

2.4 Discussion

The findings of the present study demonstrate that irrespective of acute or chronic dosing, ginkgo administered alone leads to an increase in deoxy-Hb as compared to placebo in the pre-frontal cortex, during performance of the Stroop and 3-back tasks. Further analysis of each task over time revealed that this effect was maintained continuously for the duration of both the Stroop task and the 3-back task, and also occurred at individual epochs during the RVIP task.

In terms of the effects on cerebro-electrical activity, an acute dose of ginkgo in isolation led to an increase in frontal delta and right parietal beta activity and a reduction in frontal beta activity as compared to placebo. However, the chronic dose led to an increase in frontal and left occipital delta activity only. The acute dose of ginkgo/ginseng

combination treatment resulted in an increase in frontal and parietal delta activity, an increase in frontal and right parietal theta activity and a decrease in frontal beta activity as compared to placebo. The chronic dose of the ginkgo/ginseng combination led to an increase in frontal delta, and right fronto-parietal beta activity as compared to placebo.

In relation to the effects on cognition, the only finding was that of a significant increase in the number of RVIP false alarms following the acute dose of the ginkgo/ginseng combination treatment.

Overall and irrespective of treatment, the pattern of haemodynamic change (a task-related increase in oxy-Hb and decrease in deoxy-Hb as compared to resting baseline) is in line with previous NIRS research involving both the Stroop and N-back tasks (Hoshi, 2003; Schroeter et al., 2002). As previously discussed, an expected pattern of haemodynamic effects during cognitive task performance includes a 2-3 fold increase in oxy-Hb with a reduction in deoxy-Hb in areas of cortical activation, as blood flow exceeds the rate at which oxygen is utilised. However, the main treatment-related finding with regards the haemodynamic response in the present study, was the significant increase in deoxy-Hb following ginkgo as compared to placebo during cognitive task performance. This effect was initially observed at individual epochs within the last 3 minutes of the RVIP task and was then sustained across epochs for the duration of the Stroop and 3-back tasks. It is plausible that this reflects not simply an effect of task, but as an effect of time, since the task order was not randomised and participants completed the tasks at the same time, in the same order. The onset of the significant effect would represent a time-point of approximately 2.5 hours post-dose, behavioural research has documented improved speed of attention at this time-point and at 4 and 6 hours post-dose (Elsabagh et al., 2005; Kennedy et al., 2000). Indeed, here the significant effect was maintained until the end of testing at approximately 2hours 50 minutes post-dose. In the presence of an increase in total-Hb, an increase in deoxy-Hb has in previous interventional NIRS research, been taken to be indicative of increased oxygen extraction following resveratrol (Kennedy, Wightman, et al., 2010), and vinpocetine administration (Bonoczk et al., 2002). However,

in the present study, the task-related increase in deoxy-Hb occurred in the absence of any task-related changes to oxy-Hb or total-Hb. With these previous findings in mind it is therefore less clear if the increase in deoxy-Hb seen here reflects a similar, task-related increase in oxygen extraction. Having said this, the absence of a concomitant treatment related increase in oxy-Hb/total-Hb during task performance may simply reflect a state whereby improved extraction of oxygen means there is less of a requirement to increase blood flow.

Perhaps a more likely explanation, however, for the absence of a treatment related effect during task performance is that an increase in oxy-Hb or total-Hb had already occurred. Continuous wave NIRS (CW-NIRS) (as used in the current thesis) only measures acute changes in cerebral haemodynamics during a single, continuous recording session. This is because the concentration change data it generates is baseline-adjusted to the concentration reading taken immediately before the first data point in the recording session. It therefore cannot be used to quantify gross changes in CBF parameters that take place between two separate recording sessions. In the present study NIRS recordings began approximately 2.5 hours post-dose (and there was no baseline measure in the absence of treatment). It is possible therefore, that an effect of treatment had already occurred in oxy-Hb/total-Hb before baseline measures were taken and there was no additional increase in these parameters in response to tasks. Instead, as perhaps reflected by the observed increase in deoxy-Hb, there was an increase in metabolism.

In terms of cerebro-electrical effects, the most consistent findings were those of increased frontal delta activity following acute and chronic administration of ginkgo alone and the ginkgo ginseng combination. There was also a corresponding reduction in frontal beta activity following both treatments after the acute dose only. Interestingly the 200 mg dose of ginkgo administered here did not lead to a significant modulation of alpha activity. Previous studies have observed changes in alpha waves (whether an increase or a decrease) following acute and chronic ginkgo supplementation, in healthy populations (Itil et al., 1996), those displaying age-associated cognitive impairments (Gessner et al., 1985)

and dementia patients (Itil et al., 1998). Kunkel (1993), however, found that in a group of healthy young males, aged 24-29 years (mean age, 27 years - a cohort that more closely reflects the one used in the present study) alpha frequency and power was the least affected after 3 days supplementation with either 40, 60 or 160 mg ginkgo. Furthermore, in a group of healthy volunteers aged 19-39 years (mean age 26 years) the observed modulation of alpha following 360 mg ginkgo, failed to reach significance (Kennedy et al., 2003). This may suggest that a younger, healthy cohort may not be as susceptible to modulation in alpha activity as a result of ginkgo supplementation. An increase in delta (slow waves) in conjunction with a reduction in alpha activity (fast waves) has previously been associated with a decrease in vigilance (Itil et al., 1996). Although no change in alpha activity was observed here, alongside the observed increase in frontal delta, there was a corresponding decrease in beta activity (fast waves), following both treatments. Reduced frontal beta activity following the acute dose of both treatment conditions, is consistent with that of Kennedy et al. (2003) who demonstrated a significant reduction in eyes closed beta activity following separate doses of 360 mg ginkgo and 200 mg ginseng. Following chronic administration, however, the pattern of reduced beta activity was lost for both treatments, to be replaced following ginkgo/ginseng combination with an increase in beta activity. In terms of the effects on delta activity, although a previous, acute assessment of this combination in healthy middle aged cohorts observed a significant increase in delta activity in central, parietal and occipital positions, no significant changes in frontal delta activity were observed (Dimpfel et al., 2006). Similarly Kennedy et al. (2003) observed increases in theta activity but did not observe any changes in delta activity in healthy young adults following acute supplementation of both extracts in isolation.

In relation to performance on the RVIP task, the finding of an increased number of false alarms following an acute dose of the combination treatment is consistent with the decrement in speed of attention observed previously following a combination of these 2 treatments (Kennedy et al., 2001a). Furthermore, it is one that is not observed following each treatment in isolation (Kennedy et al., 2004; Kennedy et al., 2000). When

considered in conjunction with neurophysiological effects, the negative impact upon behavioural performance following the combination treatment occurred in the absence of any change in deoxy-Hb. Interestingly, following ginkgo supplementation where a significant increase in deoxy-Hb was observed at individual epochs during the RVIP, there was no decrement in behavioural performance on this task. There is a possibility that the increase in deoxy-Hb seen following ginkgo, protected against the decrement in performance observed following the combination treatment. However, this explanation does not translate into significant positive behavioural effects during the Stroop and 3-back tasks where the increase in deoxy-Hb was maintained for the duration of each task, despite the suggestion that this may reflect an increase in metabolism, or evidence of increased oxygen extraction.

Interestingly there were no additional effects of taking ginkgo or the ginkgo/ginseng combination treatment for an extended 14-day period as compared to those of an acute dose. An intervention period of 2 weeks was selected since (apart from acute administration), 14 days' supplementation has been the minimum duration for eliciting either a behavioural or neurophysiological effect following chronic or sub-chronic dosing. In terms of haemodynamic effects during task performance, there were no changes observed as a result of the administration of either treatment following the chronic dose. There was also an absence of behavioural effects following chronic supplementation of both treatments. In terms of EEG, it could also be suggested (although only tentatively as no direct comparisons were made) that the chronic profile following each treatment was similar, if not marginally less marked, to that of the acute. Although studies assessing the chronic effects of these two treatments in combination are few in number, assessments of chronic ginkgo or ginseng supplementation in isolation have generally reported both behavioural (Dangelo et al., 1986; Mix & Crews, 2000, 2002; Silberstein et al., 2011; Sorensen & Sonne, 1996; Stough et al., 2001) and physiological effects (Santos et al., 2003; Silberstein et al., 2011) following a minimum of 14 days supplementation. However, it is important to point out that in the present study, the chronic effects of treatment were measured in the presence of an acute dose.

Consequently, it is difficult to definitively state that any chronic effects observed are truly those of a chronic dose alone, as they may reflect those of an acute dose superimposed on those of a chronic.

There were, however, limitations to the present study, in particular the absence of a pre-treatment resting baseline session to obtain NIRS measurements and cognitive task performance data in the absence of treatment. This was because doing so would have required participants to remain seated and connected to the NIRS device throughout the absorption period, for up to 3 hours prior to the cognitive assessment, which was unfortunately not viable. The significance of this is that gross changes in haemodynamic parameters as a result of treatment administration could not be measured, due to the absence of a pre-treatment, resting baseline. It should also be noted that since integration of the methods of EEG and NIRS could not be achieved they had to be implemented separately. Owing to the time-consuming nature of EEG set-up ('capping-up'), within the present study, EEG was implemented first so that set-up could occur during the absorption period. This therefore makes it difficult to compare findings from each assessment, in order to make overall conclusions as the observed electrical activity was recorded up to 1 hour prior to the modulation of cerebral oxygenation. This also leads to the possibility that some early effects of treatment on task-related haemodynamics could have been missed, since NIRS recordings did not occur until ~2.5 hours post-dose. Future studies would benefit from better understanding of how NIRS and EEG technologies could be implemented in unison via their integration. This would allow a true parallel assessment of cerebro-electrical and cerebral haemodynamic effects during task performance.

In conclusion, the current study has demonstrated that an acute 207mg dose of ginkgo leads to significant increase in deoxy-Hb during performance of tasks that activate the pre-frontal cortex and that both ginkgo and a ginkgo/ginseng combination modulate cerebro-electrical activity following both acute and chronic supplementation.

Chapter 3: The acute effects of two doses of *Ginkgo biloba* on cerebral blood flow, cognitive performance and mood in healthy adults.

3.1 Introduction

Chapter 2 established that a ~200 mg dose of ginkgo is capable of augmenting the haemodynamic changes in cerebral oxygenation parameters, in this case deoxy-Hb, caused by the performance of cognitive tasks in healthy young adults. However, it also highlighted that the absence of a pre-treatment NIRS baseline was a methodological limitation that needed to be addressed, since without it there could be no measure of gross changes in cerebral oxygenation/blood flow as a result of treatment.

Previous research of ginkgo's cerebro-vascular effects in healthy older adults, has demonstrated that chronic supplementation of 80 mg *Ginkgo biloba* results in an increase in cerebral perfusion (Santos et al., 2003). A 120 mg dose has led to an increase in cerebral blood flow (Mashayekh et al., 2011), and a shift from bilateral activation to right dominant activation during a memory task (Sakatani et al., 2014). As a consequence of the limited research into the cerebral blood flow effects of ginkgo supplementation in healthy young populations, and with the most interesting findings from chapter 2 being those on cerebral oxygenation following ginkgo alone (as opposed to being combined with ginseng), it was evident that these effects needed to be explored further and more thoroughly within this population. This would be achieved by evaluating the cognitive and cerebral blood flow effects of *Ginkgo biloba* in isolation within a healthy young population, at different doses, following acute supplementation. Since the findings of chapter 2 did not reveal any effects specific to the chronic dose, it was therefore decided to include an acute assessment of dose only.

As previously discussed, cognition research has documented that ginkgo, at a range of doses, is capable of modulating performance in healthy young volunteers. For example, it has led to positive effects on a number of tasks, including memory, attention and speed of attention as well as improving subjective feelings of alertness and

contentment (Elsabagh et al., 2005; Kennedy, Jackson, et al., 2007; Kennedy et al., 2000, 2002). The results from the previous chapter have demonstrated that a 207 mg dose of *Ginkgo biloba* is capable of increasing deoxy-Hb in response to task performance in a healthy young population. The present study aimed to expand on this research by determining if this finding would extend to different doses, namely 180 mg and 360 mg. It also aimed to address the methodological limitations of the previous chapter, by including a pre-treatment NIRS baseline measure, to determine if gross changes in cerebral oxygenation would be also be observed at these doses. From a series of previous assessments of ginkgo supplementation, as described above, the 360 mg dose has been identified as the most beneficial in terms of its acute effects on cognition and mood in healthy young populations (Kennedy et al., 2002). Neurophysiological research of this dose, however, is lacking. In contrast, in healthy young populations, 180 mg is a dose that has rarely been assessed in acute ginkgo research for either its impact on cognition or neurophysiological effects. This is despite a number of studies choosing to assess the physiological and cognitive effects of doses ranging around this level, including 120 mg, 200 mg and 240 mg (Elsabagh et al., 2005; Itil et al., 1996; Jezova, Duncko, Lassanova, Kriska, & Moncek, 2002; Kennedy, Jackson, et al., 2007; Kennedy et al., 2003; Kennedy et al., 2000).

It was anticipated in chapter 2 that, based on ginkgo's previously documented ability to modulate blood flow, there would be some evidence of this effect following ginkgo alone or when administered in combination with ginseng (see chapter 2). Methodological issues of this initial study aside (for example, the absence of a pre-treatment NIRS baseline), an alternative explanation for ginkgo's lack of effects in chapter 2, may be because the young healthy population used were, by their nature, already performing close to the peak of their abilities. Therefore the demands of the tasks may not have been high enough to detect an effect of treatment. Indeed, in populations where cognitive and physiological processes are undermined and there exists 'room for improvement' (such as in those with age-related cognitive decline or clinical conditions normally associated with ageing), ginkgo has received a degree of attention in terms of

cognitive and vascular research. This is largely due to the predominantly positive effects it is capable of eliciting (Mashayekh et al., 2011; Santos et al., 2003; Winther, Randlov, Rein, & Mehlsen, 1998; Wu et al., 2008). Perhaps therefore in young, healthy populations, a higher level of demand is required in order to more clearly observe any effects of treatment, this could be generated by manipulating the level of difficulty required of the tasks and increasing the level of mental fatigue experienced as a result. It is anticipated that increasing the difficulty of the tasks would limit ceiling effects and create an environment where the identification of any effects as a result of treatment are more apparent. Similarly, the increased demands of the tasks would elevate CBF (which ginkgo has been shown to facilitate), which in turn would mean that any shortfalls induced by limited substrates, would be reduced. Cognition research in healthy young adults has demonstrated that increased availability of metabolic substrates can lead to measurable improvements in cognitive performance (Kennedy & Scholey, 2000; Moss et al., 1998; Scholey et al., 2001), in particular when task demand is high (Kennedy & Scholey, 2000; Scholey et al., 2001). With this in mind, a manipulation of task demand through the repeated use of the most subjectively difficult tasks may lead to a sensitive backdrop from which to identify any beneficial effects of ginkgo in a healthy young population. Chapter 2 has verified that NIRS is able to detect specific, task-related changes in deoxy-Hb following ginkgo administration and previous research has established the ability of NIRS to identify changes in cerebral oxygenation as a result of increasing task demand (Izzetoglu et al., 2004; Izzetoglu et al., 2003; Shibuya-Tayoshi et al., 2007).

There are a limited number of studies to date that have assessed the influence of *Ginkgo biloba* on cerebral blood flow during the performance of cognitive tasks, despite the herbal extracts separately documented, cognitive (Elsabagh et al., 2005; Kennedy, Jackson, et al., 2007; Kennedy et al., 2000, 2002) and vascular effects (Galduroz, Antunes, & Santos, 2007; Sakatani et al., 2014; Santos et al., 2003). The most probable reason for this being that the majority of methods for assessing cerebral blood flow are restrictive and the range of tasks it is possible to administer whilst implementing these methods are limited. The present study will therefore address this gap in literature by

administering a range cognitively demanding tasks whilst CBF is measured via NIRS following acute ginkgo administration of 2 different doses.

The findings of chapter 2 demonstrated that there were limitations in terms of the methodology adopted. The inclusion of a pre-treatment baseline measurement for cognitive performance as well as a resting pre-treatment baseline for NIRS assessment is also particularly important. This will clearly define any on-treatment, task-related effects and delineate any changes in cerebral oxygenation/CBF as a result of treatment, over time. A testing schedule that begins within and not from 2 hours of treatment administration is also necessary in order to ensure any early treatment effects are captured. Ginkgo supplementation has demonstrated vascular, cerebro-electrical and cognitive effects from as early as 30/60 minutes post-dose (Itil et al., 1996; Jezova et al., 2002; Kennedy, Jackson, et al., 2007; Kennedy et al., 2000, 2001b). A shorter, 90-minute absorption period also provides the advantage of creating a less demanding schedule for the participant. This is particularly relevant here since the inclusion of a pre-treatment NIRS baseline would mean participants would not be able leave their seat from arrival until the end of testing, a time which would total ~2.5-3 hours. In line with this, the EEG assessment included in chapter 2 was removed in order to focus on the cerebro-vascular effects of the extract. Although there are studies that have successfully combined these methods to provide simultaneous EEG and NIRS measurements (Fazli et al., 2012; Roche-Labarbe et al., 2008; Wallois et al., 2012), the time necessary and equipment required for their successful integration in the current project was not available. Given that NIRS permits assessments in a wider range of scenarios than EEG, and due to the observed changes in cerebral oxygenation as a result of ginkgo supplementation in chapter 2, it was felt that this measure would provide the most informative results in the current study. With regards to the tasks administered, a similar group was employed to those in chapter 2 (including serial 7s, 3-back and RVIP); however, with the inclusion of more mental arithmetic tasks (serial 13s, 17s). This feature, in combination with the repetition of the tasks 6 times in succession, meant this battery was therefore deemed to

be harder. The inclusion of blood pressure and heart rate measurements were intended to provide a measure of the peripheral effects of acute ginkgo supplementation.

The aim of the present placebo-controlled, double blind, crossover study was therefore to determine the acute cerebro-vascular, cognitive and mood effects of two doses (180 mg and 360 mg) of ginkgo. It would also assess if the repeated use of tasks that are more demanding and fatiguing will lead to a more pronounced physiological and behavioural cognitive effect in a young, healthy population.

3.2 Method

3.2.1 Participants

Twenty-four healthy young participants (9 males, 15 females) between the ages of 18 and 27 (mean age 21.9, SD 2.74; BMI 22.7, SD 2.7) were recruited. The study was approved by the Northumbria University, Division of Psychology and Sport Sciences ethics committee and conducted in accordance with the Declaration of Helsinki. Prior to participation, volunteers were required to sign an informed consent form, see appendix A for example of information sheet provided. A general health screen (see appendix B) informed volunteers that they would not be eligible to take part if they had a history of neurological, vascular or psychiatric illness, a history or current diagnosis of drug or alcohol abuse, a current diagnosis of depression or anxiety, anaemia, high blood pressure, a heart or respiratory disorder, type 1 diabetes or phenylketonuria. They would also not be eligible if they had a history of head trauma, migraines, learning difficulties, dyslexia or attention deficit hyperactivity disorder (ADHD). All participants reported that they were in good health, had normal or corrected-to-normal vision and had no known allergies to the treatment ingredients. Additionally, they were not currently taking any dietary supplements and were free from any ‘over the counter’, herbal or prescribed medications, with the exception, for some female participants, of the contraceptive pill. Female volunteers also reported that they were not pregnant or seeking to become pregnant. Habitual smokers consuming more than three cigarettes per day were excluded from the

study. Since testing was taking place in the afternoon, participants who reported drinking >6 cups of coffee per day (or the equivalent in caffeine from other sources) were excluded. From the self-report caffeine consumption questionnaire (see appendix C), participants reported consuming caffeine on a daily basis, but <6 cups (range 64 mg-382 mg per day).

3.2.2 Design and treatment

A randomised, double-blind, counter-balanced, within subjects, placebo-controlled design was utilised. Participants attended 3 study visits and at each received 1 of the following treatments: 180 mg *Ginkgo biloba* LI 1370, a standardised extract containing 25 % total ginkgo flavonoids and 6 % percent total terpene lactones (Lichtwer Healthcare GmbH & Co, Germany); 360 mg *Ginkgo biloba* LI 1370, or placebo. Each treatment was administered in the form of 4 capsules in order to mask any taste differences and ensure participants remained blind to the treatment they had received. The order in which participants received each treatment was determined by Latin square.

3.2.3 Physiological, cognitive and mood measures

3.2.3.1 Near-infrared spectroscopy measurements

Please see chapter 2 for a description of the NIRS method used, which is identical to that used in the present chapter.

3.2.3.2 Cognitive and mood measures

All cognitive and mood measures were delivered using COMPASS. Please see chapter 2 for a description. The tasks were chosen based on their ability to activate the pre-frontal cortex (Cohen et al., 1997; Drummond et al., 1999; Lawrence et al., 2002) or their sensitivity to *Ginkgo biloba* (Kennedy et al., 2002). Tasks were also chosen based on their identification previously as being the most cognitively demanding tasks within COMPASS (Wightman, 2013). All baseline and post-dose tasks lasted 2 minutes and were presented in the following order; serial 7s subtractions, serial 13s subtractions, serial

17s subtractions, RVIP and N-back (3-back). Prior to the completion of baseline tasks and following the completion of post-dose tasks, participants were required to complete a subjective assessment of their mood via the Bond Lader visual analogue scales and a subjective mental fatigue rating scale. Tasks completed at baseline and post-dose were identical and followed the same order. At baseline each task was completed once and at post-dose each set of tasks was completed 6 times.

3.2.3.2.1 Serial 7s:

Please see chapter 2 for a description of this task. This task was scored for number of correct responses and number of errors.

3.2.3.2.2 Serial 13s:

The serial 13s task was identical to the serial 3s task as described in chapter 2, except that it involved serial subtraction of 13s. This task was scored for number of correct responses and number of errors.

3.2.3.2.3 Serial 17s:

The serial 17s task was identical to the serial 3s task as described in chapter 2, except that it involved serial subtraction of 17s. This task was scored for number of correct responses and number of errors.

3.2.3.2.4 RVIP:

Please see chapter 2 for a description of this task. This task was scored for percentage of target strings correctly detected, average reaction time for correct detections, and number of false alarms.

3.2.3.2.5 3-Back:

Please see chapter 2 for a description of this task. This task was scored for percentage of correct responses and reaction time.

3.2.3.2.6 Subjective mental fatigue visual analogue scale:

Please see chapter 2 for a description of this task.

3.2.3.2.7 Bond lader visual analogue scales:

The Bond Lader Visual Analogue Scales (Bond & Lader, 1974) consist of a series of 16 antonyms assessing aspects of mood (e.g. Calm-Excited, Alert-Drowsy, Interested-Bored, etc.) which anchor a 100 mm scale. In the current study the antonyms were presented to the participants on-screen and they were required to respond by using a mouse to click at a point on the line which best described how they felt in relation to the relevant antonym. The participants' answers were then combined to form three mood factors as recommended by the authors: alertness, calmness and contentment. These analogue scales have previously been shown to be sensitive to *Ginkgo biloba* (Kennedy, Haskell, Mauri, & Scholey, 2007; Kennedy et al., 2002).

3.2.3.3 Blood pressure and heart rate

Blood pressure and heart rate were monitored using the Boso-Medicus Prestige (Bosch + Sohn, Germany), an automatic device that provides measures of heart rate (bpm) and systolic and diastolic blood pressure (mmHg). Readings were taken from the upper left arm following 5 minutes seated rest at each visit upon arrival and following post-dose completion of the cognitive tasks.

3.2.4 Procedure

Participants were required to attend the laboratory on 4 separate occasions. The first visit was a screening session and participants were informed about the nature of the study, its requirements and its restrictions. Informed consent was obtained and their eligibility to participate was confirmed. Habitual caffeine intake and source were assessed via questionnaire (see appendix II) and familiarisation with the tasks to be administered on the study days was conducted. The remaining 3 study visits were identical, apart from the treatment subjects received. On these days, participants attended the lab at 1.30 pm after abstaining from alcohol from 8 pm the previous evening and following a 2 hour fast where they were permitted to only drink water. Prior to the 2 hour fast they were required to

consume the same light breakfast (toast or cereal was suggested) on each study day. If they were caffeine consumers they were required to consume their usual caffeinated beverage with breakfast, at least 2 hours prior to their study session, to reduce the possibility of overnight caffeine withdrawal impacting upon cognitive or physiological parameters. Upon arrival at the laboratory, heart rate and blood pressure readings were taken and baseline mood and mental fatigue scales were completed. Participants were then provided with a light snack which they had 10 minutes to consume and consisted of a choice of either a plain cheese or plain ham sandwich and a glass of water. The snack provided was the same at each visit. The NIRS headband was then fitted, recording began and readings were allowed to stabilise before baseline performance for the day was determined on cognitive tasks. Upon completion of the tasks participants were required to sit quietly for 10 minutes whilst the pre-treatment resting NIRS baseline readings were taken. Participants were then required to take their treatment for the day. Ninety minutes post-dose (during which time participants kept the NIRS headband on and watched a non-stimulating home improvement DVD), participants completed a further 6 sets of the cognitive tasks. They then had their blood pressure and heart rate measured for a second time and a second set of mood and mental fatigue scales were completed (see Fig.3.1. for more details of procedure and task duration). Participants returned for their next study visit following (at least) a 7-day washout period.

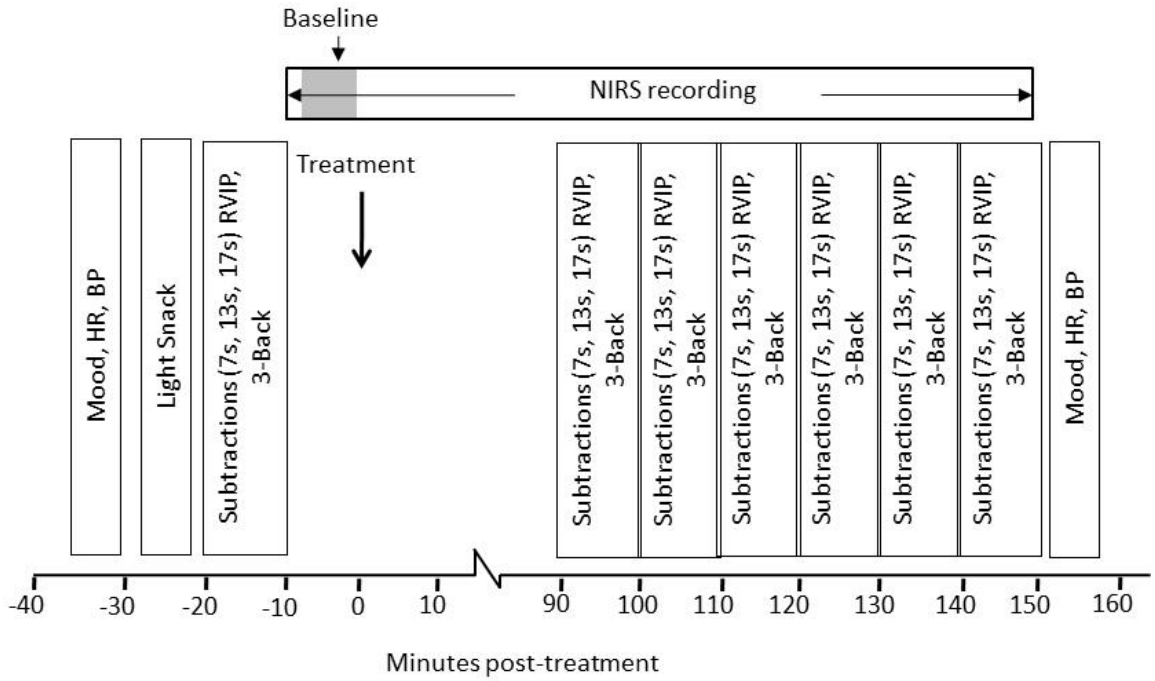


Fig.3.1. Timeline representing flow of study day.

3.2.5 Statistics

Prior to the primary NIRS analysis a within subjects ANOVA was carried out with left/right optode included as a factor to examine any treatment related hemispheric differences in response. As there were no interpretable interactions involving this factor the data from the 2 channels were averaged for the analysis.

For the primary NIRS analysis, the question under investigation was how *Ginkgo biloba* would modulate the haemodynamic response over time, during the course of the study visit (throughout the absorption period and during overall, individual task performance) in comparison to placebo. Data for oxy-Hb, deoxy-Hb and total-Hb was averaged across 10 minute (absorption) and 2 minute (individual task) epochs and baseline adjusted to the post-task resting pre-treatment period. It was then analysed by 2-way repeated measures ANOVA (treatment (180 mg *G. biloba*, 360 mg *G. biloba* or placebo) X epoch (39). Significant treatment related interactions were then described with reference to *a priori* planned comparisons, where each active treatment was compared to placebo at each epoch utilising t-tests calculated with the Mean Squares Error from the

ANOVA (Keppel, 1991). In order to reduce the potential for Type I errors only those planned comparisons associated with a statistically significant difference on the initial ANOVA are reported. In addition, only those instances where a consistent pattern of significant differences are maintained across epochs are identified as reportable significant effects.

In order to explore the haemodynamic effects of individual task performance, without influence of the absorption period and to also to allow any effects of demand (experienced through continued repetition of the tasks) to be revealed, a further analysis was conducted on NIRS data during tasks with repetition included as a factor. Analysis of the task period was conducted by 3-way repeated measures ANOVA (treatment (as above) X task (serial 7s, serial 13s, serial 17s, RVIP, 3-back) X repetition (6). Planned comparisons were conducted as per primary analysis, documented above.

Secondary analysis of the NIRS data was aimed at identifying the course of treatment-related haemodynamic effects within each task. Therefore, NIRS data for each task was averaged across 10 second epochs and baseline adjusted to the post-task resting pre-treatment period. It was then analysed by 3-way repeated measures ANOVA (treatment (as per primary analysis) X 10 second Epoch (60) X repetition (6). Planned comparisons were conducted as per primary analysis, documented above.

To assess the possibility of any on-day differences in cognitive performance, mood and autonomic measures at baseline, 1-way repeated measures ANOVAs were conducted on baseline data.

Cognitive performance, subjective mood, heart rate and blood pressure data were analysed as ‘change from baseline’ by two-way repeated measures ANOVA (treatment X repetition) with planned comparisons conducted as above.

3.3 Results

3.3.1 Near infrared spectroscopy

3.3.1.1 Primary analysis

Effects of treatment on cerebral blood flow over time.

3.3.1.1.1 Oxygenated haemoglobin

There were no treatment-related differences in oxy-Hb, see fig. 3.2a.

3.3.1.1.2 Deoxygenated haemoglobin

There were no treatment-related differences in deoxy-Hb, see fig.3.2b.

3.3.1.1.3 Total haemoglobin

There were no treatment-related differences in total-Hb, see fig 3.3.

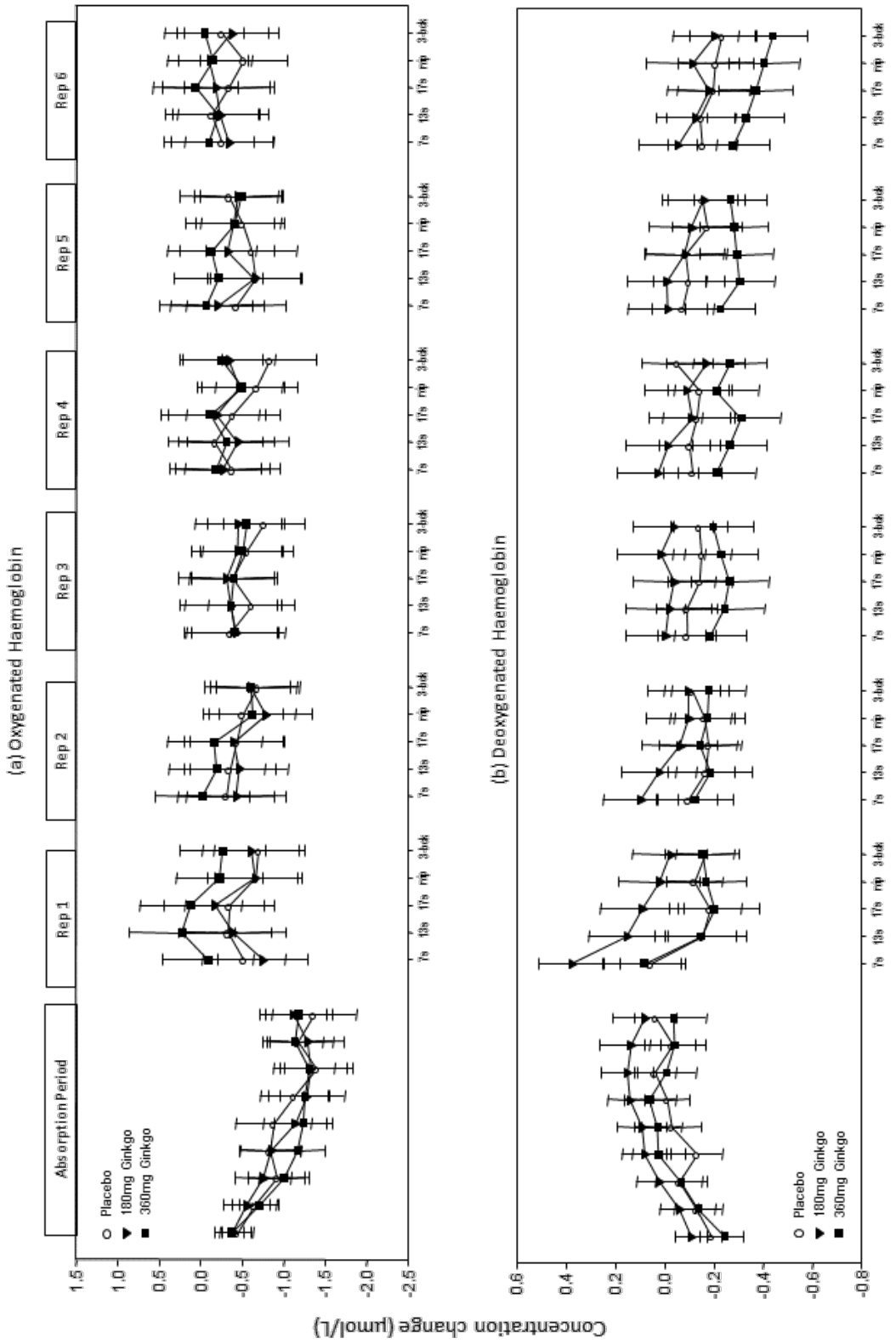


Fig. 3.2. Concentration changes of oxy-Hb (a) and deoxy-Hb (b) represented in 10 minute epochs during absorption period and 2 minute epochs during cognitive tasks following placebo, 180 mg *G. biloba*, 360 mg *G. biloba*. Means and SEM are presented as change from pre-treatment, resting baseline.

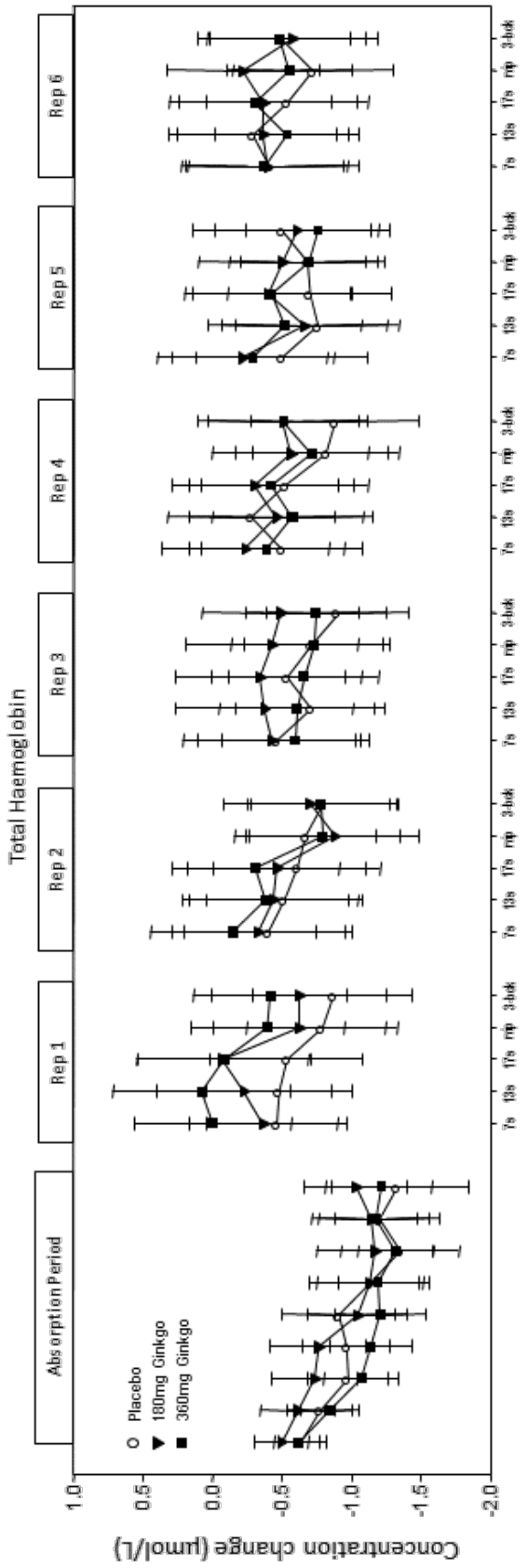


Fig. 3.3. Concentration change of total-Hb, represented in 10 minute epochs during absorption period and 2 minute epochs during cognitive tasks following placebo, 180 mg *G. biloba*, 360 mg *G. biloba*. Means and SEM are presented as change from pre-treatment, resting baseline.

3.3.1.1.4 Further primary analysis

Effects of treatment on cerebral blood flow during performance of individual tasks.

3.3.1.1.4.1 Oxygenated haemoglobin

There were no treatment-related differences in oxy-Hb, see fig. 3.4.

3.3.1.1.4.2 Deoxygenated haemoglobin

A significant interaction effect (treatment X task) was observed for deoxy-Hb [$F(8, 920)=2.02$, $p=0.046$]. Planned comparisons revealed that 180 mg ginkgo led to a significant increase in deoxy-Hb during the serial 7s [$t(920)=2.83$, $p=0.0047$, $d=-0.24$], and serial 13s subtractions tasks [$t(920)=2.41$, $p=0.0162$, $d=-0.19$], as compared to placebo. However, the 360 mg dose of ginkgo led to a significant reduction in deoxy-Hb during the serial 13s [$t(920)=2.26$, $p=0.024$, $d=0.18$], serial 17s [$t(920)=2.08$, $p=0.038$, $d=0.16$] and 3-back [$t(920)=2.03$, $p=0.043$, $d=0.17$] tasks, as compared to placebo, see fig. 3.5.

3.3.1.1.4.3 Total haemoglobin

There were no treatment-related differences in total-Hb.

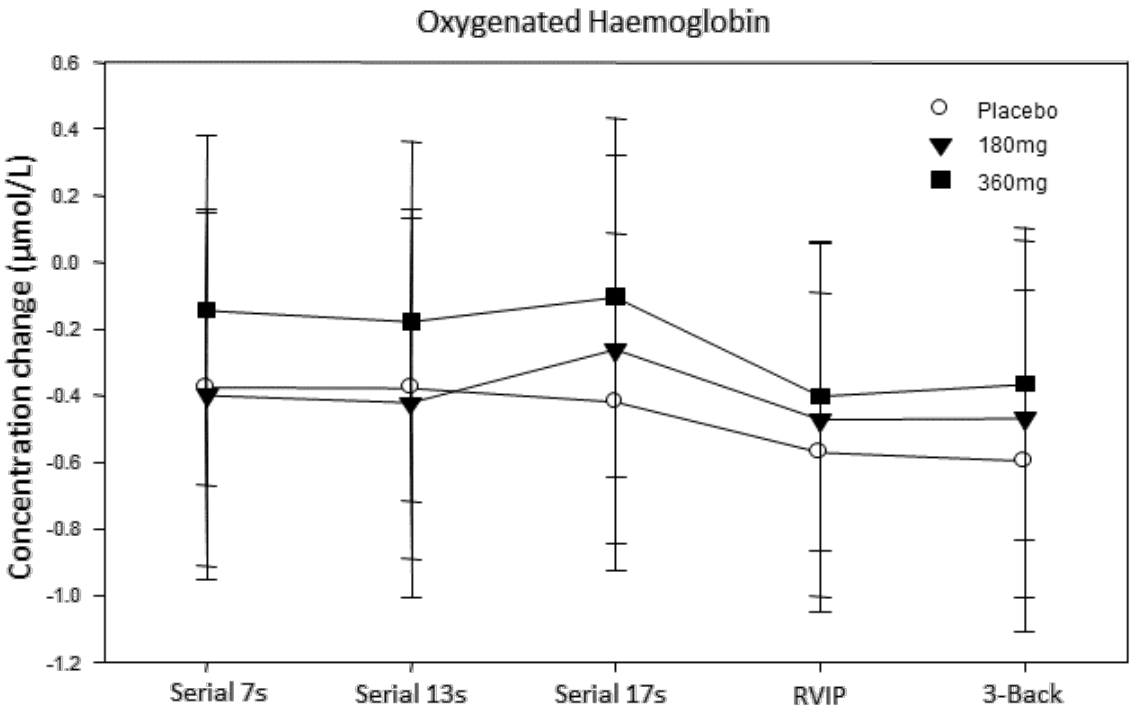


Fig. 3.4. Concentration change of oxy-Hb during each individual task following placebo, 180 mg *G. biloba*, 360 mg *G. biloba*. Means and SEM are presented as change from pre-treatment, resting baseline.

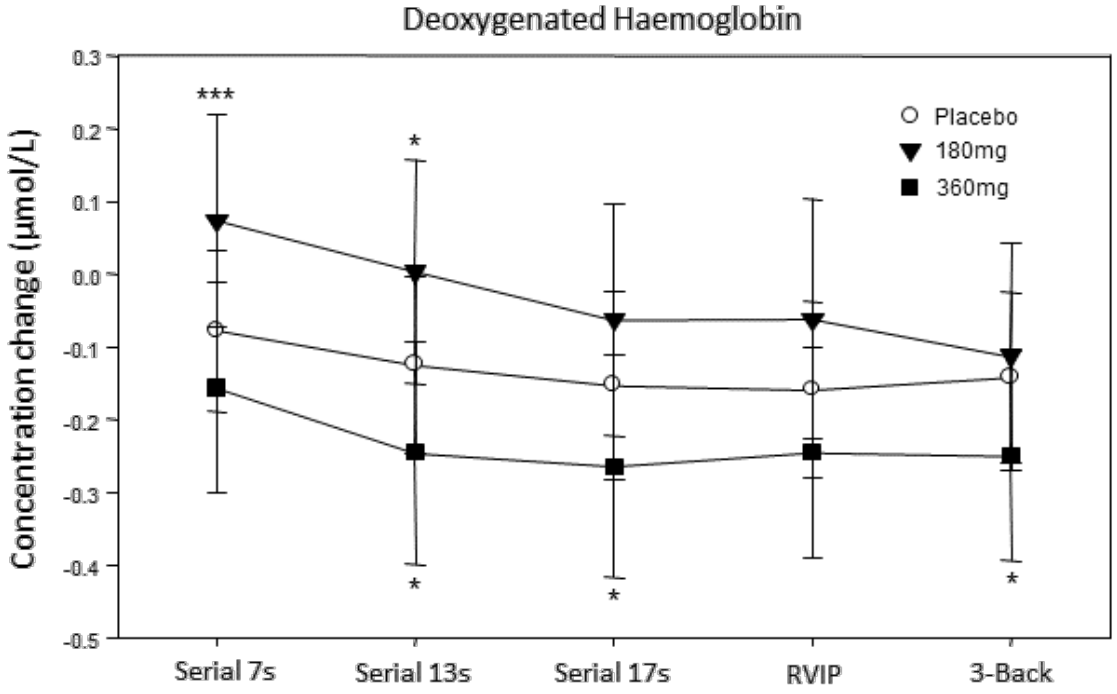


Fig 3.5. Concentration change of deoxy-Hb during each individual task following placebo, 180 mg *G. biloba*, 360 mg *G. biloba*. Means and SEM are presented as change from pre-treatment, resting baseline. Treatment X task interaction effects are shown across all reps. Significance is compared to placebo (t-tests calculated with the Mean Squares Error from the ANOVA) (* $p<0.05$, *** $p<0.005$).

3.3.1.2 Secondary analysis

Effects of treatment on cerebral blood flow during task performance using smaller duration (10 second) epochs.

3.3.1.2.1 Oxygenated haemoglobin

There were no treatment-related differences in oxy-Hb.

3.3.1.2.2 Deoxygenated haemoglobin

There were no treatment-related differences in deoxy-Hb.

3.3.1.2.3 Total haemoglobin

There were no treatment-related differences in total-Hb.

3.3.2 Cognitive performance and mood

3.3.2.1 Serial 13s

Due to incorrect task performance, only 23 participants are included in this analysis. There was a significant main effect of treatment on serial 13s subtractions errors [$F(2, 220)=3.62$, $p=0.035$]. Planned comparisons revealed that significantly fewer errors were made overall, following 180 mg ginkgo as compared to placebo [$t(220)=2.00$, $p=0.046$, $d=0.67$], see fig. 3.6.

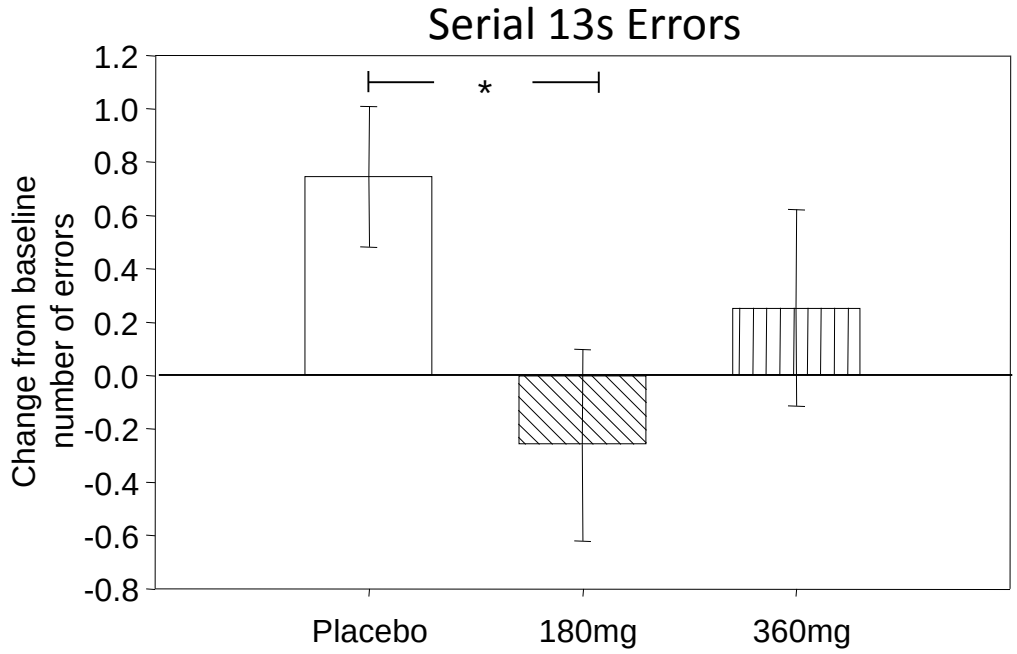


Fig. 3.6. Mean (and SEM) change from baseline scores on serial 13s subtractions errors, following 180 mg Ginkgo, 360 mg Ginkgo and placebo. Significance is compared to placebo (t-tests calculated with the Mean Squares Error from the ANOVA) (* $p<0.05$).

3.3.3 Blood pressure and heart rate

3.3.3.1 Systolic blood pressure

There were no significant differences in systolic blood pressure.

3.3.3.2 Diastolic blood pressure

There were no significant differences in diastolic blood pressure.

3.3.3.3 Heart rate

There were no significant differences in heart rate.

Table 3.1. Baseline and change from baseline scores for serial subtractions, RVIP and 3-back tasks for each treatment. Means ±SEM values are presented with F and p values from the primary ANOVA of treatment effects and treatment x repetition interactions. Significant measures are shown in bold.

Measure	*n*	Treat	Baseline	Post-dose change from baseline score: Repetition 1	2	3	4	5	6	Treat effect	Treat x rep interaction
Serial 7s subs correct (number)	24	Pla	24.50±1.57	0.92±0.10	0.42±1.07	1.29±1.02	-0.42±1.26	0.67±1.38	0.08±1.48	F<1	F<1
		180mg	24.88±1.64	-0.46±1.03	-0.50±0.84	-0.12±0.99	-1.58±1.17	0.17±1.25	-0.92±1.09		
		360mg	24.88±2.05	0.00±0.96	-0.29±1.11	-1.62±1.26	-0.92±1.39	0.87±1.10	-0.08±1.31		
Serial 7s subs errors (number)	24	Pla	1.54±0.32	-0.25±0.45	0.29±0.48	-0.21±0.37	1.00±0.57	0.38±0.49	0.67±0.45	F<1	F=1.25 p>0.1
		180mg	1.29±0.32	0.04±0.46	0.00±0.41	1.17±0.46	0.96±0.53	0.50±0.56	0.88±0.52		
		360mg	1.21±0.23	0.13±0.33	0.54±0.29	1.46±0.41	1.21±0.52	0.92±0.39	0.54±0.56		
Serial 13s subs correct (number)	23	Pla	16.61±1.37	0.91±0.82	0.91±0.61	0.70±1.19	0.65±1.03	0.13±1.18	1.22±1.13	F<1	F=1.22 p>0.1
		180mg	16.91±1.26	0.04±0.59	0.78±0.84	-1.26±1.16	0.17±0.75	1.09±0.70	0.13±0.64		
		360mg	17.09±1.57	0.04±0.91	-1.17±0.93	0.04±0.54	0.61±0.65	0.26±1.09	0.35±0.83		
Serial 13s subs errors (number)	23	Pla	1.30±0.30	0.30±0.44	0.48±0.40	0.78±0.44	0.96±0.42	1.13±0.43	0.83±0.38	**F=3.62 p<0.05**	F<1
		180mg	2.09±0.37	-0.43±0.37	-0.61±0.42	-0.09±0.64	0.04±0.54	-0.35±0.43	-0.13±0.47		
		360mg	1.74±0.40	-0.39±0.51	0.22±0.39	0.13±0.37	0.48±0.58	0.39±0.61	0.70±0.51		
Serial 17s subs correct (number)	24	Pla	12.33±0.90	0.96±0.67	0.75±0.61	1.83±0.65	1.58±0.67	1.92±0.65	2.25±0.61	F<1	F<1
		180mg	12.00±1.16	1.17±0.87	1.58±0.66	1.58±0.89	2.12±0.87	2.25±0.82	2.54±0.86		
		360mg	12.42±0.87	1.67±0.60	0.71±0.71	0.54±0.54	0.50±0.73	1.88±0.78	2.63±0.72		
Serial 17s subs errors (number)	24	Pla	1.96±0.42	-0.46±0.41	-0.67±0.38	-0.33±0.43	0.04±0.39	-0.13±0.37	-0.17±0.48	F=1.75 p>0.1	F<1
		180mg	2.21±0.34	-0.54±0.40	-0.08±0.37	-0.50±0.35	0.25±0.49	0.17±0.61	-0.38±0.39		
		360mg	1.54±0.32	0.08±0.36	0.75±0.52	0.75±0.40	0.79±0.41	0.42±0.35	0.25±0.44		
RVIP correct (%)	23	Pla	59.78±3.98	6.79±3.31	0.00±2.89	0.00±3.14	0.27±2.34	-3.80±3.46	-3.80±3.26	F=1.08 p>0.1	F<1
		180mg	63.59±3.57	-0.40±3.46	-4.08±3.53	-2.17±3.35	-7.61±4.04	-11.4±4.30	-10.3±3.03		
		360mg	63.04±3.24	-0.27±3.80	-2.45±3.66	-7.88±4.33	-6.52±4.11	-6.52±4.54	-4.62±4.97		
RVIP RT (ms)	23	Pla	514.7±15.6	-13.5±9.94	-1.93±11.9	-11.9±10.8	-8.43±9.69	8.07±12.2	-12.8±14.5	F=1.59 p>0.1	F=1.42 p>0.1
		180mg	491.8±10.2	9.12±8.19	12.6±12.1	12.6±11.5	15.4±11.9	-1.22±10.7	10.3±10.9		
		360mg	485.8±10.2	1.30±6.69	14.7±7.66	9.68±10.9	29.3±9.58	31.0±9.50	5.08±11.6		
RVIP false alarms (number)	23	Pla	1.43±0.31	0.26±0.28	0.00±0.34	0.13±0.28	-0.39±0.29	-0.13±0.32	0.09±0.33	F=1.48 p>0.1	F<1
		180mg	1.13±0.22	0.43±0.40	0.22±0.24	0.39±0.33	0.78±0.34	0.57±0.26	0.26±0.30		
		360mg	1.52±0.31	-0.26±0.36	-0.39±0.34	-0.04±0.34	0.26±0.34	0.17±0.42	0.00±0.52		
3-back accuracy (%)	24	Pla	92.96±1.27	-0.74±1.17	-0.37±0.95	-0.09±1.03	-1.39±1.44	-0.93±1.27	-1.48±1.16	F<1	F<1
		180mg	92.78±1.50	0.00±0.98	-1.85±1.37	-0.65±1.35	-1.39±1.17	-2.32±1.31	0.55±1.20		
		360mg	91.58±1.48	0.55±1.41	-0.09±1.47	-0.56±1.63	-2.41±1.49	0.09±1.46	-1.39±1.29		
3-back RT (ms)	24	Pla	1659±195.0	-166±65.2	-251±83.4	-338±116	-373±125	-483±150	-526±167	F<1	F=1.24 p>0.1
		180mg	1388±125.0	-122±55.1	-183±66.5	-211±64.1	-186±75.3	-282±77.7	-390±77.4		
		360mg	1463±147.9	-192±71.2	-305±96.5	-213±105	-203±88.1	-300±101	-338±122		

Table 3.2. Change from baseline scores for derived measures from Bond Lader and mental fatigue visual analogue scales for each treatment. Means ±SEM values are presented with F and p values from the primary ANOVA of treatment effects and treatment x repetition interactions. Significant measures are shown in bold.

Measure		*n*	Treat	Baseline	Post-dose change from baseline score	Treatment effect
Mental fatigue (mm)		24	Placebo	34.75±2.86	19.0±4.87	F<1
			180mg	38.00±2.47	18.2±3.29	
			360mg	40.71±3.08	17.0±3.75	
Bond-Lader factors (mm)	Alert	24	Placebo	61.65±2.07	-13.3±3.69	F<1
			180mg	60.23±1.85	-13.3±2.63	
			360mg	58.47±2.31	-10.9±3.71	
	Content	24	Placebo	65.15±1.84	-6.14±2.52	F<1
			180mg	64.20±1.48	-6.77±2.72	
			360mg	65.33±1.76	-4.08±2.00	
	Calm	24	Placebo	55.67±2.48	1.48±2.46	F=1.29 p>0.1
			180mg	56.08±2.76	5.98±2.73	
			360mg	61.13±2.64	2.00±2.32	

3.4 Discussion

The current study has demonstrated that irrespective of task repetition, 180 mg ginkgo, as compared to placebo, leads to a significant increase in deoxy-Hb during serial 7s and serial 13s subtraction tasks. Whereas a 360 mg dose of ginkgo leads to a significant reduction in deoxy-Hb as compared to placebo during serial 13s, serial 17s and 3-back tasks.

In terms of cognitive effects, the only significant finding was that of a reduction in the number of serial 13s errors following the 180 mg dose of ginkgo as compared to placebo.

In relation to the effects of ginkgo on cerebral oxygenation, in the previous chapter although ginkgo did not modulate oxy-Hb, a 207 mg dose led to a significant increase in deoxy-Hb as compared to placebo, for the duration of the Stroop and 3-back tasks as well as the RVIP task, but to a lesser extent. It is interesting therefore that in the present study an increase in deoxy-Hb was only observed for the lower, 180 mg dose and only during the serial 7s and serial 13s tasks. The 360 mg dose in contrast, evinced a significant reduction in deoxy-Hb as compared to placebo during the serial 13s task, which also extended to the serial 17s and 3-back tasks. Why two doses of the same treatment should engender contrasting effects on the same cerebral oxygenation parameter is not readily apparent. A decrease in deoxy-Hb with a corresponding increase in oxy-Hb, in the absence of any intervention, is generally accepted to be indicative of brain activation (Obrig & Villringer, 2003). When considered in relation to the effects following 360 mg ginkgo (see fig. 3.5), although there was no significant effect on oxy-Hb here, the pattern of observed effects is suggestive of an increase as compared to placebo (see fig. 3.4). Turning to the contrasting effects following the lower 180 mg dose, an increase in deoxy-Hb as a result of the administration of an intervention believed to increase cerebral blood flow (such as ginkgo) has been observed previously, as discussed in chapter 2. At rest, Bönöczk et al. (2002) demonstrated similar findings in stroke patients following treatment with vinpocetine and in healthy young adults, Kennedy et al. (2010) observed the same pattern following the administration of resveratrol. In the latter study the effects were

observed during completion of serial 7s subtractions and the RVIP task (both of which were used in the present study). In both instances this modulation of deoxy-Hb was identified as indicative of increased oxygen utilisation and extraction (Bonoczk et al., 2002). Again, as discussed in chapter 2, the aforementioned studies also observed associated increases in oxy-Hb and total-Hb, which represent an increase in oxygen delivery and total blood flow, and are thought to occur in the presence of increased neural demand (Izzetoglu et al., 2004; Izzetoglu et al., 2003; Shibuya-Tayoshi et al., 2007). This may therefore suggest that during certain tasks, following a ~200 mg dose of ginkgo, an increase in oxygen extraction and utilisation occurs in the absence of an increase in demand for neural substrates.

The absence of an increase in cerebral blood flow in the present study could, perhaps, be explained by the dosing schedule in addition to the study population used. Santos et al. (2003) found that cerebral perfusion was significantly increased in frontal, parietal and occipital areas of the brain in healthy older adults (60-70 years) following 8 months administration of an 80 mg/day dose of a standardised ginkgo extract. Similarly Mashayekh et al. (2011) also documented an increase in cerebral blood flow in healthy older adults (51-71 years) following 4 weeks supplementation of 120 mg/day with a standardised dose of ginkgo. However, the effects were milder than those of Santos et al. (2003) and only observed in the left parietal-occipital area of the brain. It is possible that the shorter, 4 week dosing schedule administered by Mashayekh et al. (2011) was responsible, at least in part, for the difference in magnitude of the cerebral blood flow effects between the two studies. Therefore, in terms of the present study, this may suggest that the acute dosing regimen used here was not sufficient to elicit an effect. In terms of the study population used, both of the aforementioned studies were conducted in older cohorts and cerebral blood flow is known to reduce with age (Leenders et al., 1990; Martin et al., 1991; Pantano et al., 1984). With this in mind, it could be that increases in cerebral blood flow as a result of ginkgo administration are not as readily identifiable in younger cohorts as they are in ageing populations where blood flow may be sub-optimal. Furthermore, the repeated use of difficult tasks employed in the present study to increase

cognitive demand, were perhaps not difficult or fatiguing enough to provide the room for improvement needed to observe an effect of treatment in a young population. It is unfortunate therefore that no mental fatigue scales were included after the competition of each set of tasks, as this could have verified the level of mental fatigue the participants were experiencing. It is also of interest to note that in the present study following both treatments as well as placebo, there was a reduction in oxy-Hb during the performance of cognitive tasks as compared to the resting baseline. NIRS is capable of detecting cerebral activation during the completion of cognitively demanding tasks and as previously discussed, this is commonly expressed as an in increase in oxy-Hb with a corresponding decrease in deoxy-Hb. However, in the current study, this pattern of effects was not observed for oxy-Hb, most pointedly where no intervention was used (i.e. following placebo). One possibility is that this was due to falsely elevated oxy-Hb readings since measurement of the resting NIRS baseline occurred immediately after participants had completed their baseline cognitive assessment.

The only finding in terms of behaviour was that of a reduction in serial 13s errors following the 180 mg dose, leading to the possibility that it was in fact, a type 1 error. On the other hand, it could be said to be as expected, as ginkgo has elicited the same effects following a similar version of the same task (serial 3s), previously (Kennedy et al., 2002). However, it is interesting that it was the only cognitive/mood parameter to evince any benefit of ginkgo, when a 360 mg dose has previously led to an increase in serial 7s responses as well as an increase in alertness and contentedness using the same task and mood scales as in the present study (Kennedy et al., 2002). However, a key difference between the present study and that of Kennedy et al. (2002) is that here participants were required to complete the tasks 6 times in succession, creating a more demanding environment where treatment related effects may have been lost. The relative lack of cognitive effects is unfortunate but not entirely unexpected. Although there have been a number of instances where similar doses to those used here have elucidated significant results, the effects from study to study have not always been consistent, despite the use of similar tasks and methodologies. Although the treatment used was a

standardised extract, the means and method of processing such extracts can vary between manufacturers (Van Beek, 2002) a factor which may have had an influence upon the outcomes. A further explanation for the lack of effects in the current study may have been the time at which testing took place. Ginkgo has been reported as reaching its peak in the blood at 2.3 hours post-dose (Drago, Floriddia, Cro, & Giuffrida, 2002); however, in the present study testing began at 1.5 hours and only continued until 2.5 hours post-dose. This raises the possibility that any cognitive effects ginkgo may have engendered were missed by premature cessation of the post-dose testing period. This proposal seems more likely considering that previously observed improvements in speed of attention (Kennedy et al., 2000, 2002) and mental arithmetic (Kennedy et al., 2002) were first observed at 2.5 and 4 hours respectively and then at 6 hours post-dose despite initial testing taking place 1 hour following treatment.

Turning to autonomic measures, the absence of an effect on blood pressure and heart rate following acute administration of ginkgo has been documented previously (Jung, Mrowietz, Kiesewetter, & Wenzel, 1990; Keheyan, Dunn, & Hall, 2011). However, a stress induced reduction in blood pressure has been reported following 120 mg ginkgo (Jezova et al., 2002). Even though no specific stressor was applied in the present study, a testing battery such as the one administered here (difficult tasks re-administered 6 times over the period of an hour) could reasonably be regarded as a situation likely to induce a degree of stress. Therefore, it is interesting that no effect of ginkgo was demonstrated here, particularly in view of ginkgo's previously documented effects on blood flow and blood circulation.

In terms of limitations, one of the most identifiable in the present study could be regarded as the duration of the post-dose testing window. As previously discussed, ginkgo's effects have been demonstrated as early as 1 hour and up until 6 hours post-dose; however, the present study was only able to observe the effects within 1.5-2.5 hours of administration. The main reason for this being, that once the NIRS equipment has been placed upon the participant's head, it cannot be removed, due to the way in which

NIRS measures are taken (NIRS provides concentration change data from first reading prior to start of recording). Therefore, expecting participants to sit for a period of up to 4 or indeed 6 hours at a time without the chance of a break or rest would be unreasonable and likely lead to a high drop-out rate. An alternative approach would be to use an intervention that had either a relatively quick absorption period and or a comparatively short psychoactive/biologically active phase, when using NIRS technology.

In conclusion, the current research has demonstrated that *Ginkgo biloba* is capable of reducing the number of errors made on a demanding task of mental arithmetic as well as modulating deoxy-Hb, but that these effects are dependent upon the dose administered. In addition, this study has gone further to demonstrate that NIRS is sensitive enough to identify treatment-related changes in cerebral oxygenation parameters during the performance of demanding cognitive tasks.

Chapter 4: An evaluation of the effects of caffeine and L-theanine both alone and in combination on cerebral haemodynamics, cognitive performance and mood.

4.1 Introduction

The findings of chapters 2 and 3 demonstrated to an extent, that NIRS is able to detect changes in cerebral oxygenation parameters during the performance of cognitive tasks, in the presence of herbal extracts known for their vasodilatory properties. However, in order to remove some of the uncertainty experienced in chapters 2 and 3 in terms of how the intervention would modulate behavioural and physiological parameters; it seemed prudent to administer an intervention with more robust behavioural and vascular effects, in this case, caffeine. It was anticipated that this would provide the platform for a more thorough evaluation of the methodological approach adopted within this thesis. Additionally, since chapters 2 and 3 evaluated the effects of interventions with supposed vasodilating properties, the administration of caffeine - an intervention known for its opposing (vasoconstricting) effects on blood flow, also seemed appropriate. The beneficial effects of caffeine on cognition and mood have been reported in a number of studies; however, relatively few studies have looked at the effects of caffeine in combination with other compounds (see Haskell et al. (2013) for review), despite the fact that caffeine is seldom consumed in isolation. L-theanine is a naturally occurring amino-acid that is found almost uniquely in tea *(Camellia sinensis*), where it coexists with caffeine and has been used for centuries in Asia in order to produce feelings of relaxation (Heese et al., 2009). Although studies of the behavioural and peripheral effects of these two interventions combined have reported synergistic effects, the impact on cerebral blood flow of this combination remains unexplored.

Caffeine is the most widely consumed psychoactive substance in the world, with coffee and tea representing our main dietary sources (Fredholm, Battig, Holmen, Nehlig, & Zvartau, 1999b). Caffeine has been shown to reduce cerebral blood velocity as assessed by transcranial Doppler (Hasse, Becka, Kuhlmann, & Wensing, 2005) and

cerebral blood flow (CBF) as assessed by near infrared spectroscopy (NIRS) (Kennedy & Haskell, 2011) and magnetic resonance imaging (MRI) (Chen & Parrish, 2009a; Laurienti et al., 2003; Mathew & Wilson, 1991; Rack-Gomer et al., 2009). These effects on CBF have been shown to be dose-dependent following 1, 2.5 and 5 mg/kg caffeine (Chen & Parrish, 2009b). Studies that have assessed the effects of caffeine administration on blood flow taking a participant's caffeine consumption habits into consideration, have found (via quantitative perfusion MRI), that the effects are dependent upon the level of caffeine habitually consumed. Following at least 30 hours abstention, habitual high consumers were shown to exhibit increased resting CBF as compared to low consumers and caffeine use was significantly positively correlated with CBF following both placebo and caffeine (Addicott et al., 2009; Field et al., 2003). In addition, despite retaining a higher rate of CBF overall, high consumers also exhibited a greater acute reduction in CBF in response to a 250 mg dose of caffeine whilst in a state of withdrawal (Field et al., 2003). However, it should be noted that Addicott et al. (2009) found no difference between low, moderate and high consumers in response to caffeine which was administered whilst in a withdrawal state.

In studies assessing cognitive performance and mood, caffeine has consistently been shown to improve reaction times (Childs & de Wit, 2006; Haskell et al., 2008b; Smit & Rogers, 2000) and alertness (Quinlan et al., 2000; Rogers et al., 2008), irrespective of consumption status (Haskell et al., 2005).

Unlike caffeine, however, research into the effects of L-theanine in humans is limited. In terms of anxiolytic effects, 200 mg L-theanine has been shown to reduce acute stress responses (subjective perception, heart rate and salivary immunoglobulin A) induced by a mental arithmetic task (Kimura et al., 2007). A 250 mg dose of L-theanine was also found to slow reaction time on a visual probe task indicating reduced anxiety (Rogers et al., 2008). EEG studies have also provided some support for these findings with increases in resting alpha activity observed following 50 mg (Nobre et al., 2008) and 200 mg L-theanine (Juneja et al., 1999), which the authors interpret as being indicative of relaxation. A dose of 250 mg of L-theanine has also been shown to differentially alter

alpha activity during task performance and at rest (Gomez-Ramirez et al., 2007; Gomez-Ramirez et al., 2009).

In terms of cognitive function, L-theanine in isolation has been shown to engender decrements in performance (Gomez-Ramirez et al., 2007; Haskell et al., 2008b) or, at best, an absence of effects (Gomez-Ramirez et al., 2009; Haskell et al., 2008b; Kelly et al., 2008; Owen et al., 2008). However, when administered together L-theanine modulates or potentiates the effects of caffeine. For instance, Haskell et al. (2008b) reported that a number of effects were evident following a combination of 250 mg L-theanine and 150 mg caffeine that were not apparent when each treatment was administered alone. Improvements included increased speed on several tasks (simple reaction time, delayed word recognition, numeric working memory) and improved sentence verification accuracy. Participants also rated themselves as being more 'alert' and less 'tired'. In contrast, caffeine alone, but not the combination, only improved reaction times on a digit vigilance task. Other studies have employed lower doses of L-theanine (~100 mg) and caffeine (~50 mg) to explore these effects (Einother, Martens, Rycroft, & De Bruin, 2010; Kelly et al., 2008; Owen et al., 2008). These studies provide support for the findings from studies of higher doses, with the combination treatment leading to improved accuracy (Einother et al., 2010; Kelly et al., 2008; Owen et al., 2008) and speed (Einother et al., 2010; Owen et al., 2008) on tasks of attention, and improvements to measures of memory (Owen et al., 2008). L-theanine has also been shown to antagonise the physiological effects of caffeine. Rogers et al. (2008) demonstrated that although systolic and diastolic blood pressure were significantly higher following the administration of 250 mg caffeine when compared to placebo or 200 mg L-theanine alone, the combination of the two compounds led to an attenuation of this effect. There was, however, no evidence of antagonism of mood effects.

As discussed, previous research using a variety of methodologies has demonstrated that caffeine improves aspects of cognitive performance (Childs & de Wit, 2006; Haskell et al., 2008b; Haskell et al., 2005; Quinlan et al., 2000; Rogers et al., 2008; Smit & Rogers, 2000) and consistently reduces cerebral blood-flow (Chen & Parrish,

2009a; Laurienti et al., 2003; Mathew & Wilson, 1991; Rack-Gomer et al., 2009). Specifically, a previous study using the dose of caffeine employed here (75 mg) showed that caffeine significantly reduced total-Hb as measured by NIRS (Kennedy & Haskell, 2011). Given that co-administration of L-theanine with caffeine has previously been demonstrated to attenuate its haemodynamic effects (Rogers et al., 2008), it remains a possibility that co-administration may also attenuate the cerebro-vascular effect of caffeine. The doses of L-theanine/caffeine and the ratios in which they have been administered in the majority of assessments of these two interventions to date have tended to contain higher levels of L-theanine than caffeine. This is not representative of the levels or ratios normally found in tea in our diet, which tend to be in the region of 2 to 1 in favour of caffeine. Similarly, the lowest dose of L-theanine explored previously in terms of behaviour (100 mg) is equivalent to ~4 cups of tea. The doses used in the present study (75 mg caffeine, 50 mg L-theanine and a combination of 75 mg caffeine and 50 mg L-theanine) therefore more closely reflect the ratios found in tea and represent the levels found in approximately 2 cups. The decision to include both habitual and non-habitual caffeine consumers was taken based on previous research documenting differences in CBF as a result of consumer status (Addicott et al., 2009; Field et al., 2003). It would also allow assessment of the impact of status on CBF effects following caffeine/L-theanine within the present study. To be consistent with chapter 3, and specifically following findings of an antagonistic interaction between L-theanine and caffeine on this measure (Rogers, 2007), blood pressure and heart rate readings were also monitored, pre and post-treatment.

This double-blind, placebo-controlled, balanced, crossover study aimed to address these issues using NIRS to measure CBF in the pre-frontal cortex during cognitive task performance following caffeine and L-theanine both alone and in combination. The cohort included both habitual tea drinking consumers and non-habitual consumers of caffeine, and involved administration of doses and ratios of caffeine and L-theanine that more closely reflect those present in tea. It is hypothesised that administration of caffeine alone will lead to a reduction in CBF and that this effect will be attenuated when administered in

the presence of L-theanine. It is also predicted that this combination will enhance the effects of caffeine on behaviour.

4.2 Method

4.2.1 Participants

Twenty-four healthy young participants (10 males, 14 females) between the ages of 18 and 35 (mean age 21.8, SD 3.19; BMI 22.8, SD 3.1) were recruited. Volunteers were recruited to take part in the study if they fell into one of two pre-defined categories; 'habitual consumers' (those who drank tea and consumed more than 150 mg caffeine per day) or 'non-habitual consumers' (those who consumed less than 60 mg caffeine per day and no more than two cups of tea/coffee per week). Twelve participants were classified as habitual consumers (5 males; mean age 23.3, SD 3.65) and 12 as non-habitual consumers (5 males; mean age 20.4, SD 1.88). From the self-report caffeine consumption questionnaire (see appendix C), habitual consumers reported drinking between 163 mg to 432 mg caffeine per day (mean 252.2, SD 74.3). Non-habitual consumers reported drinking between 0 and 56 mg caffeine per day (mean 16.7, SD 15.6). With regards tea consumption, habitual consumers reported consuming between 1 to 6 cups per day (mean 3.50, SD 1.46) and non-habitual consumers reported consuming between 0 and 2 per week (mean 0.45, SD 0.62). The study was approved by Northumbria University's School of Psychology and Sport Sciences' ethics committee and conducted in accordance with the Declaration of Helsinki. Prior to participation, volunteers were required to sign an informed consent form (see appendix A for example of information sheet) and complete the aforementioned caffeine consumption questionnaire that assessed their daily level of caffeine intake. A general health screen (see appendix A) informed volunteers that they would not be eligible to take part if they had a history of neurological, vascular or psychiatric illness. Participants would also be excluded if they had a history or current diagnosis of drug or alcohol abuse, a current diagnosis of depression or anxiety, anaemia, high blood pressure, a heart or respiratory

disorder, type 1 diabetes, phenylketonuria, a history of head trauma, migraines, learning difficulties, dyslexia or ADHD. All participants reported that they were in good health, had normal or corrected-to-normal vision and had no known allergies to the treatment ingredients. They also reported that they were not currently taking any dietary supplements or medication, including the contraceptive pill as intake of oral contraceptives has been shown to increase the half-life of caffeine (Patwardhan, Desmond, Johnson, & Schenker, 1980). Additionally, they were not colour-blind, did not smoke and in the case of female volunteers that they were not pregnant or seeking to become pregnant.

4.2.2 Design and treatment

A double-blind, counter-balanced, within subjects, placebo-controlled design was utilised. Participants attended 4 study visits and at each received one of the following treatments: 75 mg caffeine (pharmaceutical grade caffeine powder, Blackburn Distributions Ltd); 50 mg L-theanine (pharmaceutical grade L-theanine powder, Suntheanine, Taiyo Europe, Germany); 75 mg caffeine and 50 mg of L-theanine in combination, or placebo. The doses selected roughly equate to the levels of L-theanine found in 2 cups of tea. These were chosen in an attempt to extend previous findings exploring 100 mg L-theanine (Einother et al., 2010; Kelly et al., 2008; Owen et al., 2008) whilst more closely reflecting the ratio of L-theanine to caffeine found in tea. Each treatment was administered in the form of 2 capsules in order to mask any taste differences and ensure participants remained blind to the treatment they had received. The order in which participants received each treatment was determined by Latin square and random allocation to treatment order for each group (habitual consumers and non-habitual consumers).

4.2.3 Salivary caffeine levels

Saliva samples were obtained using salivettes (Sarstedt Ltd). One sample was taken upon arrival and one immediately following the post-dose cognitive assessment. This was to ensure overnight caffeine abstinence and to confirm caffeine absorption following caffeinated treatments (no analysis of post-treatment caffeine levels were made

following placebo or L-theanine). Once taken, samples were frozen at -20ºC. The samples were then thawed and the caffeine levels in the saliva samples were measured using an Emit® Caffeine Assay (Dade Behring Ltd).

4.2.4 Physiological, cognitive and mood measures

4.2.4.1 Near infrared spectroscopy measurements

Please see chapter 2 for a full description of the NIRS method used, which is identical to that used in the present chapter.

4.2.4.2 Cognitive and mood measures

All cognitive and mood measures were delivered using COMPASS which has previously been shown to be sensitive to caffeine (Kennedy & Haskell, 2011). Please see chapter 2 for a description of COMPASS and an explanation of tasks listed here but not described in full below. The tasks were chosen based on their ability to activate the pre-frontal cortex (Drummond et al., 1999; Lawrence et al., 2002; Schroeter et al., 2002) or their known sensitivity to one or both of the nutritional interventions under investigation (Haskell et al., 2008b; Kennedy & Haskell, 2011; Lieberman, Wurtman, Emde, Roberts, & Coviella, 1987). The tasks completed at baseline and post-dose were identical with the exception of duration (all baseline tasks were 2 minutes). Tasks were presented in the following order (post-dose duration in parentheses) serial 3s subtractions (4 minutes), serial 7s subtractions (4 minutes), simple reaction time (SRT), (8 minutes), RVIP (8 minutes), choice reaction time (CRT), (8 minutes) and Stroop (8 minutes). Prior to baseline task completion and following completion of post-dose tasks participants completed a subjective assessment of their mood in the form of the caffeine research visual analogue scales.

4.2.4.2.1 Serial 3s:

Please see chapter 2 for a description of this task. This task was scored for percentage accuracy and percentage errors.

4.2.4.2.2 Serial 7s:

Please see chapter 2 for a description of this task. This task was scored for percentage accuracy and percentage errors.

4.2.4.2.3 Simple reaction time:

An upwards pointing arrow appears on the screen at a random inter-stimulus duration of between 1 and 3.5 seconds. Participants have to respond as quickly as they can when they see a stimulus appear by pressing the space bar. One hundred and ninety stimuli were presented and the task was scored for reaction time.

4.2.4.2.4 RVIP:

Please see chapter 2 for a description of this task. This task was scored for percentage of target strings correctly detected, average reaction time and percentage of false alarms.

4.2.4.2.5 Choice reaction time:

An arrow pointing either left or right is presented in the middle of the screen at a random inter-stimulus duration of between 1 and 3.5 seconds. As soon as participants see an arrow appear on the screen they are required to press the 'Z' key if the arrow is pointing left or the 'M' key if the arrow is pointing right. One hundred and eighty-five stimuli were presented and the task was scored for percentage of correct responses and reaction time.

4.2.4.2.6 Stroop:

Please see chapter 2 for a description of this task. This task was scored for percentage accuracy and reaction time.

4.2.4.2.7 Caffeine research visual analogue scales:

Caffeine research visual analogue scales adapted from Rogers et al. (2003) that have previously been used in caffeine and L-theanine research were also included (Haskell et al., 2008b; Haskell et al., 2005; Kennedy & Haskell, 2011) and were presented on-screen. Participants were shown the following descriptors "relaxed", "alert", "jittery", "tired", "tense", "headache", "overall mood", "mentally fatigued" and asked to rate how much they matched their current state by placing an 'x' on a 100 mm line with the end

points labelled ‘not at all’ (left hand end) and ‘extremely’ (right hand end), with the exception of “overall mood”, which was labelled ‘very bad’ and ‘very good’. Alert and tired, then tense and relaxed scores were combined to create respective factors of “alertness” and “tension” as recommended by the authors.

4.2.4.3 Blood pressure and heart rate

Please see chapter 3 for details of equipment used. Readings were taken from the upper left arm following 5 minutes seated rest at each visit upon arrival and following post-dose completion of the cognitive tasks.

4.2.5 Procedure

Participants were required to attend the laboratory on 5 separate occasions. The first visit was a screening session where participants were informed about the nature of the study, its requirements and its restrictions. Informed consent was obtained and their eligibility to participate was confirmed. Habitual caffeine intake and source were assessed via questionnaire and familiarisation with the tasks to be administered on the study days was conducted. The remaining 4 study visits were identical to each other, with the exception of the treatment administered. On each day, participants attended the lab at 8 am following an overnight 12-hour fast where they were permitted only to drink water. Upon arrival heart rate and blood pressure readings were taken following 5 minutes of seated rest. Following a baseline completion of the mood scales, salivary caffeine levels were taken to ensure caffeine abstinence. Following this, the NIRS headband was fitted. Participants initially sat quietly for 5 minutes, then made a baseline completion of the cognitive tasks, this was followed by a 2-minute rest period where they sat quietly. The rest period was included to reduce the haemodynamic impact of the baseline cognitive tasks upon the pre-treatment, resting baseline measure. Upon completion of the tasks participants were required to sit quietly for a 2 minute NIRS resting baseline period. Participants were then required to take their treatment for the day. Following a 30-minute absorption period (during which time NIRS recording continued whilst participants watched a non-stimulating wildlife DVD), participants completed a second set of the

cognitive tasks and a final rest period (8 minutes; included to allow an assessment of whether any CBF effects apparent after the absorption period are as a result of increased neural demand during tasks or are simply due to the time-course of effects of treatment). They then had their blood pressure and heart rate measured for a second time, rated their mood for a second time, and gave a second saliva sample, which was used to confirm caffeine absorption following caffeinated treatment (see fig 4.1 for more details of procedure and task duration). Participants returned for their next study visit within 7 days, following (at least) a 48-hour washout period.

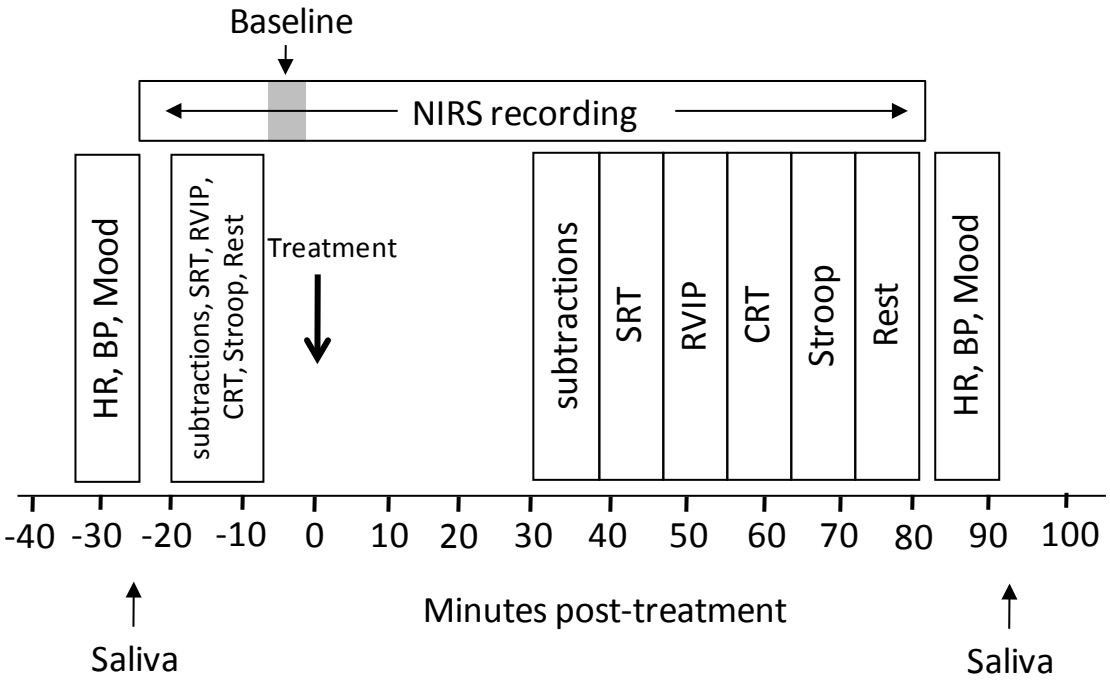


Fig 4.1. Timeline representing flow of study day.

4.2.6 Statistics

Two paired sample t-tests were utilised to analyse baseline and post-caffeinated treatment salivary caffeine levels to confirm caffeine absorption for caffeine containing treatments (caffeine, combination). To determine if any differences existed in salivary

caffeine levels following caffeine and combination treatment, 'change from baseline' data were analysed by 2-way repeated measures ANOVA (treatment X consumer status).

Prior to the primary NIRS analysis a within subjects ANOVA was carried out with left/right optode included as a factor to examine any treatment related hemispheric differences in response. As there were no interpretable interactions involving this factor the data from the two channels were averaged for the analysis.

For the primary NIRS analysis, the question under investigation was how caffeine, L-theanine and the combination treatment would modulate haemodynamics over time, during the course of the study visit (throughout the absorption period and during overall, individual task performance) in comparison to placebo and if consumer status would be a factor in this response. Data for oxy-Hb, deoxy-Hb and total-Hb was averaged across the 4 minute (absorption period) and 8 minute (individual tasks) epochs and baseline adjusted to the 2-minute post-task resting pre-treatment period. To account for disruption in NIRS readings as a result of consumption of treatment, the first 2 minutes of the absorption period was omitted from the analysis. Data was analysed by 3-way repeated measures ANOVA (treatment (75 mg caffeine, 50 mg L-theanine, 75 mg caffeine and 50 mg L-theanine in combination or placebo) X epoch (absorption period - 7 x 4 minute epochs, task period – 6 x 8 minute epochs) X consumer status (habitual consumers, non-habitual consumers)). Significant treatment related interactions were then described with reference to *a priori* planned comparisons, where each active treatment was compared to placebo at each epoch utilising t-tests calculated with the Mean Squares Error from the ANOVA (Keppel, 1991). In order to reduce the potential for Type I errors only those planned comparisons associated with a statistically significant difference on the initial ANOVA are reported. In addition, only those instances where a consistent pattern of significant differences are maintained across epochs are identified as reportable significant effects.

In order to further explore the haemodynamic effect during the task period alone, to identify if effects were specific to task performance, separate analysis of the task period

was conducted by 3-way repeated measures ANOVA (treatment (as above) X task (serial subtractions, SRT, RVIP, CRT, Stroop, rest period) X consumer status (as above). Planned comparisons were conducted as per primary analysis, documented above.

Secondary NIRS analysis was aimed at assessing the effects of treatment in more detail through the use of shorter-duration epochs and identifying any treatment-related haemodynamic effects over the first and second half of each task. NIRS data was averaged across 4 minute epochs and baseline adjusted to the 2-minute post-task resting pre-treatment period. Separate analysis of the task period was conducted by 3-way repeated measures ANOVA (treatment (as per primary analysis) X (12 x 4 minute epochs) X consumer status (as per primary analysis)). Planned comparisons were conducted as per primary analysis, documented above.

To assess the possibility of any on-day or consumer status differences in cognitive performance, mood, blood pressure and heart rate prior to treatment, 2-way repeated measures ANOVAs were conducted (treatment X consumer status) on baseline data. Any significant differences were further explored with Bonferroni corrected pairwise comparisons.

Cognitive performance, subjective mood, heart rate and blood pressure data were analysed as 'change from baseline' by 2-way repeated measures ANOVA (treatment X consumer status). Significant treatment related effects were described with reference to *a priori* planned comparisons (as above) where each active treatment was compared to placebo or, in the case of interactions, each active treatment was compared to placebo in each consumer group.

4.3 Results

4.3.1 Salivary caffeine levels

Prior to analysis, baseline scores were examined, and it was confirmed that they were in accordance with levels of caffeine that would reflect overnight caffeine abstinence.

Mean baseline values were 0.34 µg/ml, consumers and non-habitual consumers levels were 0.44 µg/ml and 0.25 µg/ml respectively and confirmed overnight abstinence [levels below 1 µg/ml have previously been reported for overnight caffeine abstinence (Evans & Griffiths, 1999)]. Following caffeinated treatment, salivary caffeine levels were 2.25 µg/ml (combination) and 2.33 µg/ml (caffeine). Analysis of the results confirmed that in comparison to baseline levels, salivary caffeine was significantly higher following caffeine [$t(23) = -8.55$, $p<0.001$] and combination treatments [$t(23) = -5.54$, $p<0.001$, (see fig. 4.2). There was no significant difference between caffeinated treatments (caffeine and combination) for salivary caffeine levels post-treatment.

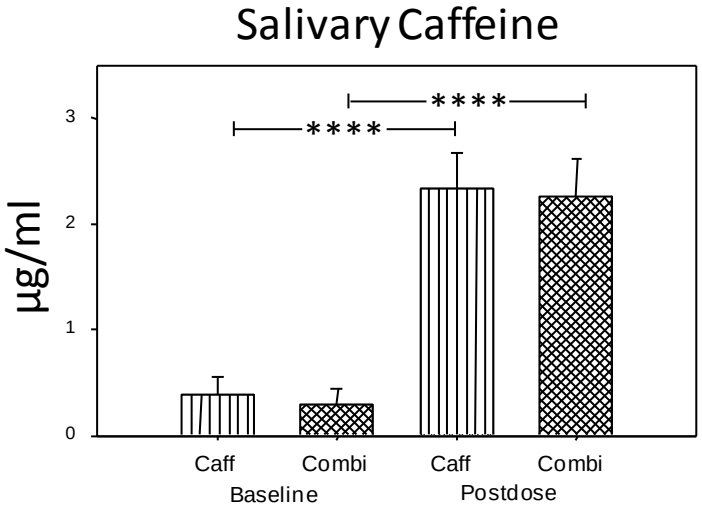


Fig 4.2. Mean (and SEM) salivary caffeine values at baseline and post-dose following a combination of 50 mg L-theanine and 75 mg caffeine and 75 mg caffeine in isolation. Paired sample t-test significance levels are shown (**** p<0.001).

4.3.2 Near infrared spectroscopy

4.3.2.1 Primary analysis

Effects of treatment on cerebral blood flow over time.

4.3.2.1.1 Oxygenated haemoglobin

A significant interaction effect (epoch X treatment) was observed for oxy-Hb [$F(36, 792)=1.50$, $p<0.05$]. Planned comparisons revealed that following caffeine as compared to placebo, oxy-Hb was significantly reduced during minutes 3-6 [$t(792)=2.05$, $p<0.05$, $d=0.32$] and 11-18 of the absorption period (minutes 11-14 [$t(792)=2.21$, $p<0.05$, $d=0.33$],

15-18 [$t(792)=2.15$, $p<0.05$, $d=0.29$]) and during SRT [$t(792)=2.67$, $p<0.01$, $d=0.31$], RVIP [$t(792)=3.63$, $p<0.001$, $d=0.38$], CRT [$t(792)=3.55$, $p<0.001$, $d=0.35$] and Stroop tasks [$t(792)=4.26$, $p<0.001$, $d=0.41$] and the rest period [$t(792)=3.98$, $p<0.001$, $d=0.44$], see fig 4.3a. This effect was not evident following L-theanine or when caffeine was combined with L-theanine, in fact, oxy-Hb was significantly increased following the combination during minutes 23-30 of the absorption period as compared to placebo (minutes 23-26 [$t(792)=2.82$, $p<0.005$, $d=-0.37$], 27-30 [$t(792)=2.19$, $p<0.05$, $d=-0.27$]), see fig. 4.3a.

4.3.2.1.2 Deoxygenated haemoglobin

In relation to deoxy-Hb, a significant interaction effect (epoch X treatment) was also evinced [$F(36, 792)=1.62$, $p<0.05$]. Planned comparisons revealed that following caffeine as compared to placebo, deoxy-Hb was significantly increased during serial subtractions [$t(792)=2.72$, $p<0.01$, $d=-0.39$], SRT [$t(792)=2.07$, $p<0.05$, $d=-0.32$], RVIP [$t(792)=2.19$, $p<0.05$, $d=-0.30$] and Stroop tasks [$t(792)=2.52$, $p<0.05$, $d=-0.31$] and during the rest period [$t(792)=2.47$, $p<0.05$, $d=-0.30$], see fig. 4.3b. Following the combination deoxy-Hb was significantly increased during serial subtractions [$t(792)=2.31$, $p<0.05$, $d=-0.36$], CRT [$t(792)=2.05$, $p<0.05$, $d=-0.30$] and Stroop tasks [$t(792)=1.97$, $p<0.05$, $d=-0.25$] as compared to placebo, see fig 4.3b. There was also a significant treatment by consumer interaction for deoxy-Hb [$F(3, 792)=3.25$, $p<0.05$]. Planned comparisons revealed that non-habitual consumers had significantly higher deoxy-Hb throughout the absorption and task periods following caffeine as compared to placebo [$t(792)=2.93$, $p<0.005$, $d=-0.85$], see fig. 4.5.

4.3.2.1.3 Total haemoglobin

There were no treatment related differences in total-Hb, see fig. 4.4.

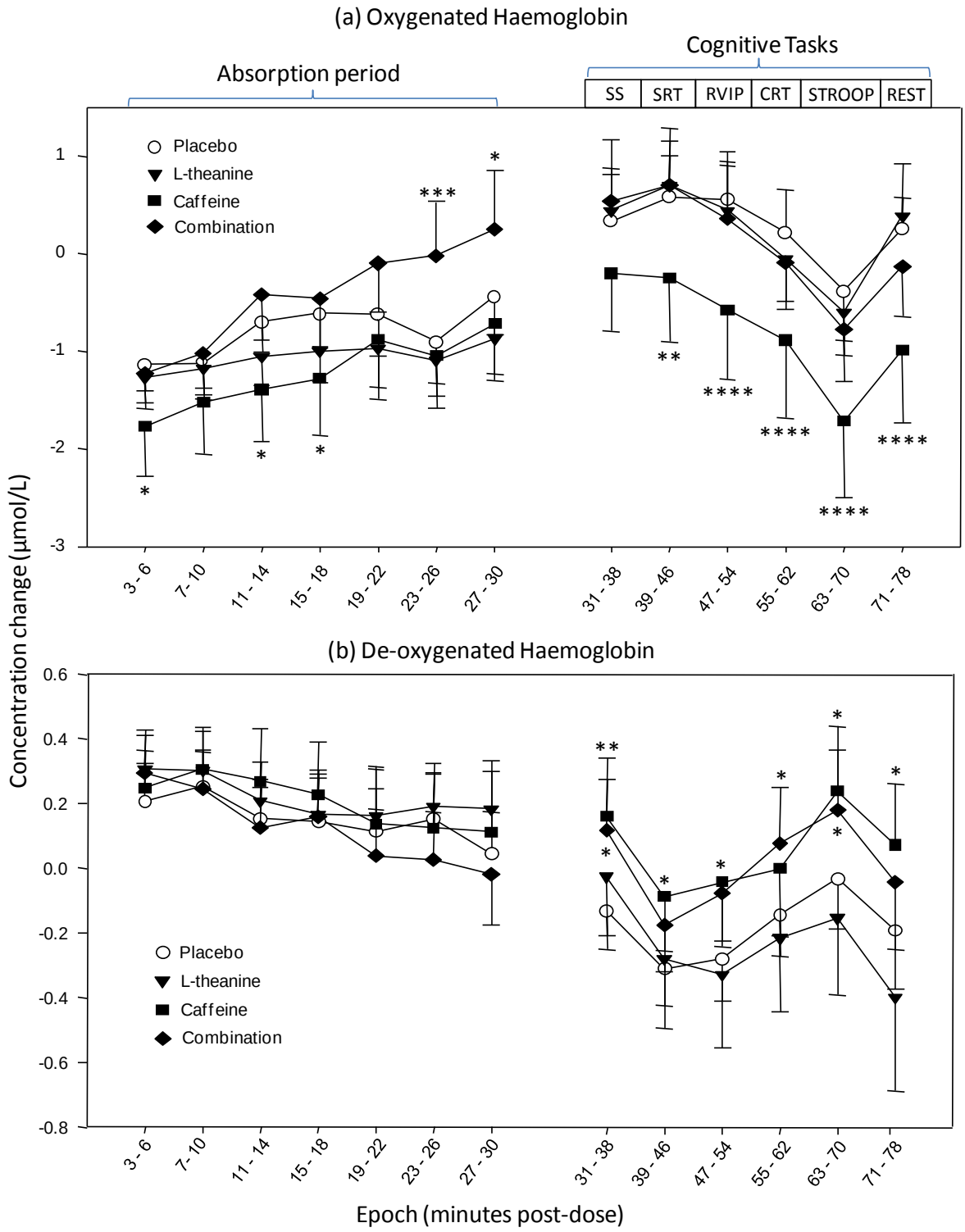


Fig. 4.3. Concentration changes of (a) oxy-Hb and (b) deoxy-Hb represented in 4 minute epochs during absorption period and 8 minute epochs during cognitive task period following placebo (○), 50 mg L-theanine (▼), 75 mg caffeine (■) and a combination of 50 mg L-theanine and 75 mg caffeine (◆). Means and SEM are presented as change from pre-treatment, resting baseline. Treatment x epoch interaction effects are shown. Significance is compared to placebo (t-tests calculated with the Mean Squares Error from the ANOVA) (* p<0.05, ** p<0.01, *** p<0.005, **** p<0.001).

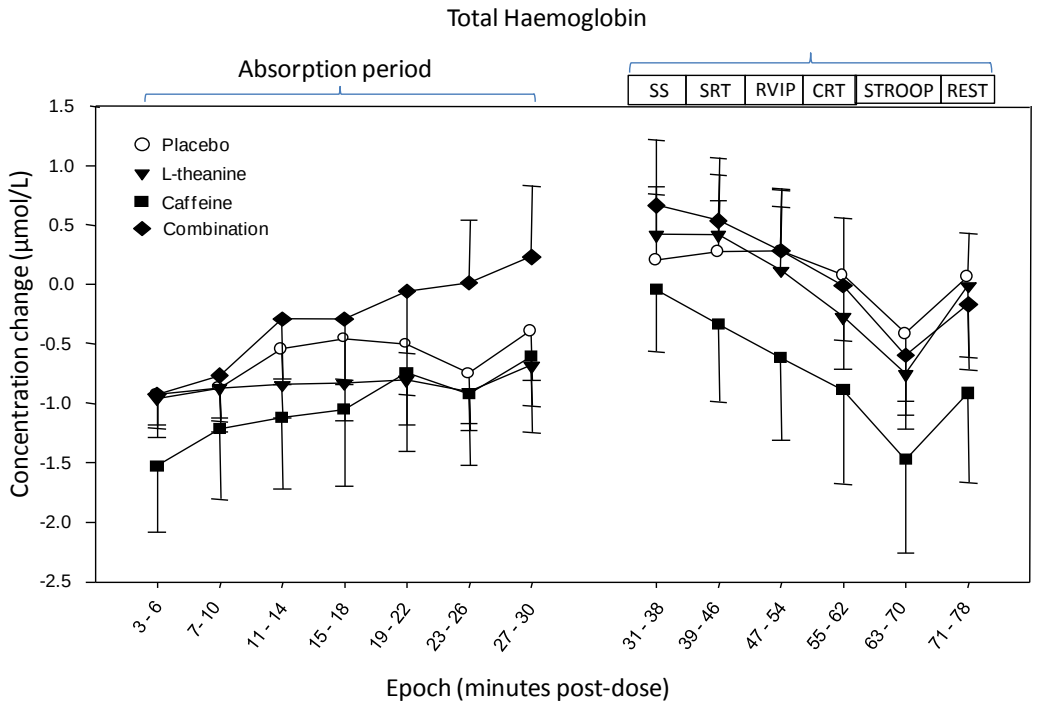


Fig. 4.4. Concentration change of total-Hb represented in 4 minute epochs during absorption period and 8 minute epochs during cognitive task period following placebo (○), 50 mg L-theanine (▼), 75 mg caffeine (■) and a combination of 50 mg L-theanine and 75 mg caffeine (◆). Means and SEM are presented as change from pre-treatment, resting baseline.

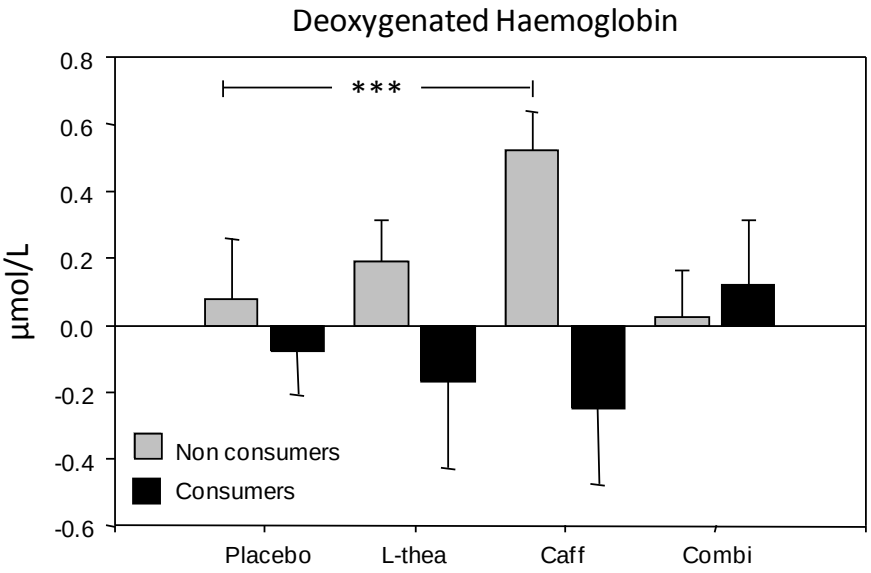


Fig. 4.5. Concentration change of deoxy-Hb overall (absorption and cognitive task period) following placebo, 50 mg L-theanine, 75 mg caffeine and a combination of 50 mg L-theanine and 75 mg caffeine. Means and SEM are presented as change from pre-treatment, resting baseline. Treatment x consumer status interaction effects are shown for deoxy-Hb (t-tests calculated with the Mean Squares Error from the ANOVA) (*** $p<0.005$).

4.3.2.1.4 Further primary analysis

Effects of treatment on cerebral blood flow during performance of individual tasks.

4.3.2.1.4.1 Oxygenated haemoglobin

There were no treatment related differences in oxy-Hb.

4.3.2.1.4.2 Deoxygenated haemoglobin

There were no treatment related differences in deoxy-Hb.

4.3.2.1.4.3 Total haemoglobin

There were no treatment related differences in total-Hb.

4.3.2.2 Secondary analysis

Effects of treatment on cerebral blood flow during task performance using smaller duration (4 minute) epochs.

4.3.2.2.1 Oxygenated haemoglobin

There were no treatment related differences in oxy-Hb.

4.3.2.2.2 Deoxygenated haemoglobin

There were no treatment related differences in deoxy-Hb.

4.3.2.2.3 Total haemoglobin

There were no treatment related differences in total-Hb.

4.3.3 Cognitive performance and mood

4.3.3.1 Baseline scores

There was a significant main effect of treatment on baseline Stroop % accuracy [$F(3, 66)=3.28$, $p<0.05$]. However, this effect was not related to placebo and was therefore not explored further. There were no other significant on-day or consumer status differences in cognitive performance, mood or autonomic measures prior to treatment.

4.3.3.2 Choice reaction time

There was a significant main effect of treatment on choice reaction time [F(3, 66)=2.99, p<0.05]. Planned comparisons revealed that reaction time was significantly faster following caffeine compared to placebo [t(66)=2.92, p<0.005, d=0.60], see fig.4.6a. However, there was also a significant treatment X consumer interaction effect [F(3, 66)=2.82, p<0.05] whereby this effect was found to be predicated on significantly faster responses in the non-habitual consumers following caffeine [t(66)=4.10, p=<0.001, d=0.97] and combination treatments [t(66)=2.30, p<0.05, d=0.62] as compared to placebo, see fig 4.6b.

4.3.3.3 Stroop

There was a significant main effect of treatment on Stroop % accuracy [F(3, 66)=5.52, p<0.005]. Planned comparisons revealed that participants were significantly more accurate following L-theanine [t(66)=2.67, p<0.01, d=-0.57] and caffeine [t(66)=3.34, p<0.005, d=-0.75] as compared to placebo, see fig 4.6c.

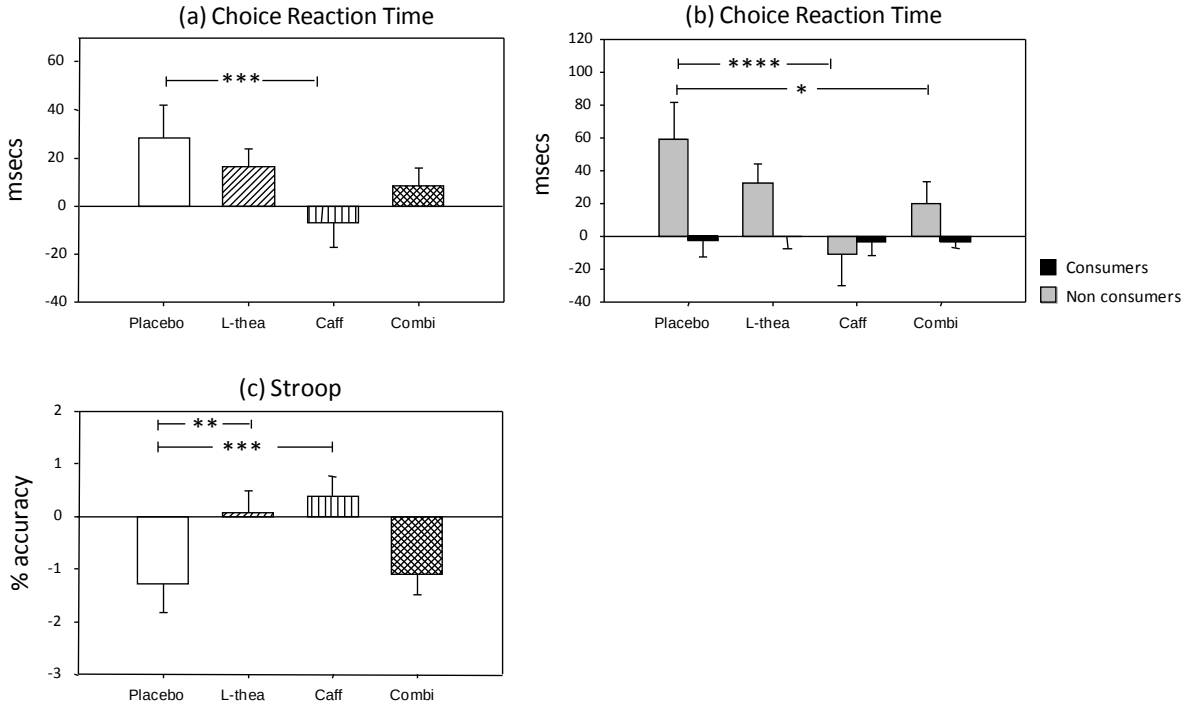


Fig. 4.6. Mean (and SEM) change from baseline scores on cognitive performance following placebo, 50 mg L-theanine (L-thea), 75 mg caffeine (Caff) and a combination of 50 mg L-theanine and 75 mg caffeine (Combi). Main effects of treatment are shown for (a) choice reaction time; interaction effects of treatment x consumer status are shown for (b) choice reaction time; main effects of treatment are shown for (c) Stroop % accuracy (* p<0.05, ** p<0.01, *** p<0.005, **** p<0.001).

4.3.3.4 'Overall mood'

There was a significant main effect of treatment on ratings of mood [$F(3, 66)=3.00$, $p<0.05$]. Planned comparisons revealed that participants' overall mood was significantly improved following caffeine in isolation as compared to placebo [$t(66)=3.00$, $p<0.005$, $d=-0.69$], see fig. 4.7a.

4.3.3.5 'Alertness' factor

There was a significant main effect of treatment on the alertness factor [$F(3, 66)=3.16$, $p<0.05$]. Planned comparisons revealed alertness was significantly increased following caffeine in isolation as compared to placebo [$t(66)=2.48$, $p<0.05$, $d=-0.47$], see fig. 4.7b.

4.3.3.6 'Tired'

The effect on alertness was largely as a result of a significant main effect of treatment on ratings of tiredness [$F(3, 66)=3.03$, $p<0.05$]. Planned comparisons revealed that participants were significantly less tired following caffeine in isolation as compared to placebo [$t(66)=2.46$, $p<0.05$, $d=0.52$], see fig. 4.7c.

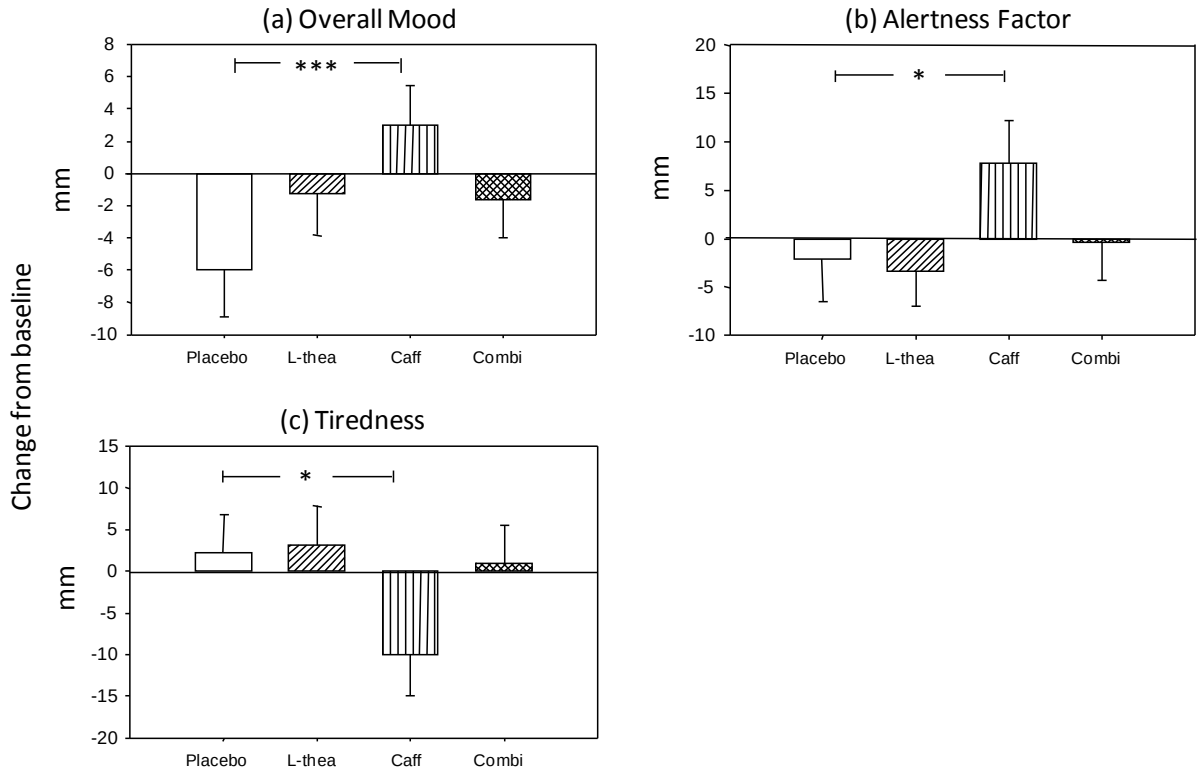


Fig. 4.7. Mean (and SEM) change from baseline scores for mood following placebo, 50 mg L-theanine (L-thea), 75 mg caffeine (Caff) and a combination of 50 mg L-theanine and 75 mg caffeine (Combi). Higher values represent more of each mood state, in the case of (a) this reflects better overall mood. Main effects of treatment are shown for (a) overall mood, (b) an alertness factor and (c) tiredness ratings (* $p<0.05$, *** $p<0.005$).

Table 4.1. Baseline and change from baseline scores for serial subtractions, SRT, RVIP and CRT tasks for each treatment. Means ± SEM values are presented with F and p values from the primary ANOVA of treatment effects and treatment x consumer interactions. Significant measures are shown in bold.

Measure	Treatment	*n*	Pre-dose baseline score		Post-dose change from baseline score		Treat effect	Treat x cons interact
			Consumers	Non-consumers	Consumers	Non-consumers		
Serial 3s subs correct (%)	Pla	12	95.41±1.54	97.07±1.26	-1.50±1.11	-1.98±2.21	**F=2.24 p>0.05**	F<1
	L-thea		95.43±1.61	95.71±1.76	0.65±0.94	-1.97±1.68		
	Caff		93.94±1.47	95.92±1.11	1.55±1.69	-0.27±1.52		
	Combi		92.36±2.09	96.30±1.10	4.15±1.67	0.50±1.40		
Serial 3s subs errors (%)	Pla	12	4.59±1.54	2.93±1.26	1.50±1.11	1.38±1.93	F<1	F<1
	L-thea		4.42±1.58	4.29±1.76	-0.50±0.85	1.74±1.82		
	Caff		6.06±1.47	4.09±1.11	-1.55±1.69	0.27±1.52		
	Combi		7.40±2.06	3.34±0.85	-3.91±1.68	-0.14±1.13		
Serial 7s subs correct (%)	Pla	12	93.30±1.48	92.18±3.55	-0.81±2.26	-0.54±4.59	F<1	F<1
	L-thea		94.31±1.79	85.54±5.82	0.45±1.83	5.31±4.14		
	Caff		92.75±3.19	90.26±3.24	1.41±3.38	-0.91±2.21		
	Combi		92.57±2.41	89.60±3.42	1.05±1.89	2.87±3.56		
Serial 7s subs errors (%)	Pla	12	6.70±1.48	7.22±3.39	0.81±2.26	1.14±4.36	F<1	F<1
	L-thea		5.69±1.79	14.46±5.82	-0.45±1.83	-5.31±4.14		
	Caff		7.25±3.19	9.19±3.18	-1.74±3.36	0.92±1.99		
	Combi		7.43±2.41	9.94±3.45	-1.05±1.89	-2.55±3.61		
SRT (RT)	Pla	12	294.00±11.19	326.54±16.59	14.39±5.59	17.55±7.89	F=1.68 p>0.1	F<1
	L-thea		288.71±11.38	329.85±19.73	19.65±8.24	35.46±7.47		
	Caff		304.90±9.96	308.08±9.96	7.31±7.40	12.12±11.00		
	Combi		290.87±9.64	322.86±24.60	13.73±6.63	17.87±11.00		
RVIP Acc (%)	Pla	12/11	70.83±6.17	62.50±4.30	-11.33±3.24	-9.80±3.90	F<1	F=1.67 p>0.1
	L-thea		68.23±5.63	67.05±4.85	-9.90±3.34	-10.37±3.20		
	Caff		75.52±7.20	62.50±6.31	-13.28±2.90	-1.56±3.67		
	Combi		68.75±8.43	67.05±5.34	-4.56±3.79	-6.82±3.58		
RVIP false alarms (%)	Pla	12/11	0.79±0.25	1.09±0.26	-0.24±0.28	-0.41±0.19	F<1	F<1
	L-thea		0.58±0.15	0.86±0.24	-0.09±0.19	0.02±0.30		
	Caff		0.42±0.12	0.96±0.25	0.01±0.12	-0.28±0.21		
	Combi		0.42±0.15	0.64±0.19	0.06±0.17	0.17±0.22		
RVIP (RT)	Pla	12/11	502.55±18.02	481.36±15.76	10.82±8.55	1.18±19.36	F<1	F<1
	L-thea		501.75±18.82	480.71±13.99	9.30±9.01	-8.83±10.44		
	Caff		510.43±18.23	487.52±12.52	2.32±7.29	-13.33±9.41		
	Combi		499.29±27.41	471.37±14.31	-0.46±13.98	-6.22±12.38		
CRT Acc (%)	Pla	12	96.17±0.87	96.67±0.79	-0.13±1.09	-0.59±0.68	F<1	F<1
	L-thea		96.33±0.69	95.33±1.68	-0.03±0.38	0.88±1.30		
	Caff		96.83±0.72	94.50±0.82	-0.44±0.64	1.76±0.49		
	Combi		96.50±0.96	95.17±1.19	0.21±0.71	1.23±0.74		
CRT (RT)	Pla	12	425.90±15.09	437.36±18.00	-2.80±9.34	59.50±22.27	**F=2.99 p=0.037**	**F=2.82 p=0.046**
	L-thea		420.64±10.14	448.00±24.75	-0.20±7.11	32.83±11.25		
	Caff		410.14±10.90	453.22±27.21	-3.30±8.44	-10.63±19.28		
	Combi		409.81±10.06	428.49±25.41	-3.10±3.84	20.06±13.08		

Table 4.2. Baseline and change from baseline scores for the Stroop task and measures derived from the caffeine research visual analogue scales for each treatment. Means ± SEM values are presented with F and p values from the primary ANOVA of treatment effects and treatment x consumer interactions. Significant measures are shown in bold.

Measure	Treatment	*n*	Pre-dose baseline score		Post-dose change from baseline score		Treat effect	Treat x cons interact
			Consumers	Non-consumers	Consumers	Non-consumers		
Stroop Acc (%)	Pla	12	98.22±0.47	97.38±0.655	-1.44±0.84	-1.12±0.71	**F=5.52 p=.002**	F<1
	L-thea	12	97.26±0.71	96.86±0.574	-0.23±0.58	0.35±0.63		
	Caff	12	96.70±1.05	95.88±1.048	0.29±0.23	0.52±0.72		
	Combi	12	97.36±1.06	97.44±0.823	-1.28±0.52	-0.93±0.57		
Stroop (RT)	Pla	12	651.67±26.36	628.92±26.501	-10.42±19.32	31.42±17.75	F=1.35 p>0.1	F<1
	L-thea	12	659.50±49.91	692.92±57.087	-22.42±33.08	-20.75±24.57		
	Caff	12	633.67±23.40	643.17±40.669	-25.83±12.89	-30.08±28.23		
	Combi	12	615.67±22.50	651.33±31.572	-10.00±15.68	-29.25±30.65		
Relaxed	Pla	12	64.75±2.73	63.83±3.643	-12.92±5.41	-8.50±4.84	F=1.49 p>0.1	F<1
	L-thea	12	55.83±5.76	63.50±4.762	0.17±5.81	-0.92±5.68		
	Caff	12	58.83±6.63	62.25±6.095	-7.33±4.38	-7.83±5.49		
	Combi	12	61.17±4.24	67.92±4.642	-6.42±8.31	-3.50±5.38		
Alert	Pla	12	57.17±5.63	50.17±6.068	-5.33±7.08	1.33±6.38	F=1.49 p>0.1	F<1
	L-thea	12	51.50±4.10	58.42±6.344	-3.58±5.35	-3.67±7.58		
	Caff	12	55.83±4.65	49.17±6.977	2.58±6.51	8.75±5.74		
	Combi	12	59.25±3.12	54.75±6.301	-2.33±4.99	3.00±7.18		
Jittery	Pla	12	28.92±5.51	17.75±3.856	8.83±6.55	5.25±5.24	**F=3.03 p=0.035**	F<1
	L-thea	12	26.92±3.59	24.25±6.273	9.17±6.65	1.67±4.77		
	Caff	12	33.33±4.32	27.17±5.328	2.92±6.18	3.75±5.78		
	Combi	12	26.50±5.26	26.58±6.405	9.17±6.82	-1.50±6.21		
Tired	Pla	12	46.25±7.87	54.25±6.703	5.42±7.06	-0.92±5.99	F<1	F=1.58 p>0.1
	L-thea	12	45.75±6.44	42.83±4.355	-0.08±6.79	6.42±6.47		
	Caff	12	50.92±5.83	53.92±6.407	-10.67±7.76	-9.25±6.46		
	Combi	12	39.08±6.37	49.67±5.875	7.75±7.23	-5.83±5.23		
Tense	Pla	12	28.50±4.27	24.42±4.892	7.67±3.51	-1.00±6.56	F<1	F=1.29 p>0.1
	L-thea	12	34.00±5.56	25.83±5.348	4.83±5.73	-2.92±5.67		
	Caff	12	32.17±4.50	26.08±6.011	2.92±5.82	3.50±7.00		
	Combi	12	31.75±6.20	18.67±3.281	1.17±3.69	6.92±4.24		
Headache	Pla	12	16.50±6.34	18.00±5.392	12.08±4.04	18.83±8.44	F<1	F=1.14 p>0.1
	L-thea	12	16.00±4.57	20.50±5.927	12.42±4.34	10.33±5.83		
	Caff	12	19.83±6.58	14.83±3.790	11.42±5.73	13.08±6.93		
	Combi	12	12.75±3.59	17.92±4.803	13.50±3.79	3.08±3.64		
Overall Mood	Pla	12	67.33±4.43	59.83±4.026	-8.00±3.23	-3.92±4.94	**F=3.00 p=0.037**	F<1
	L-thea	12	58.50±4.04	66.08±4.081	-0.17±2.66	-2.42±4.51		
	Caff	12	61.50±3.98	59.25±4.340	0.50±3.00	5.58±3.67		
	Combi	12	66.33±4.02	66.50±3.967	-5.17±2.15	1.92±3.98		
Mental Fatigue	Pla	12	35.83±7.27	30.50±5.590	16.92±7.80	17.75±7.19	F<1	F<1
	L-thea	12	39.25±5.38	35.00±5.003	14.75±6.88	11.17±6.77		
	Caff	12	29.25±5.20	33.83±6.338	15.17±7.03	11.33±8.68		
	Combi	12	30.58±6.34	31.67±6.132	14.58±5.92	5.67±5.81		

4.3.3.7 Consumer related effects

Those outcomes that demonstrated a significant between subjects effect of consumer status are reported below.

4.3.3.7.1 Serial 3s

There was a significant effect of consumer status on accuracy of serial 3s subtractions, which demonstrated that overall and irrespective of treatment, habitual consumers were significantly more accurate than non-habitual consumers [$F(1, 22)=5.45$, $p<0.05$], see fig. 4.8a.

4.3.3.7.2 Choice reaction time

There was a significant effect of consumer status on choice reaction time, which demonstrated that irrespective of treatment, habitual consumers were significantly faster than non-habitual consumers [$F(1, 22)=6.06$, $p=0.05$], see fig. 4.8b.

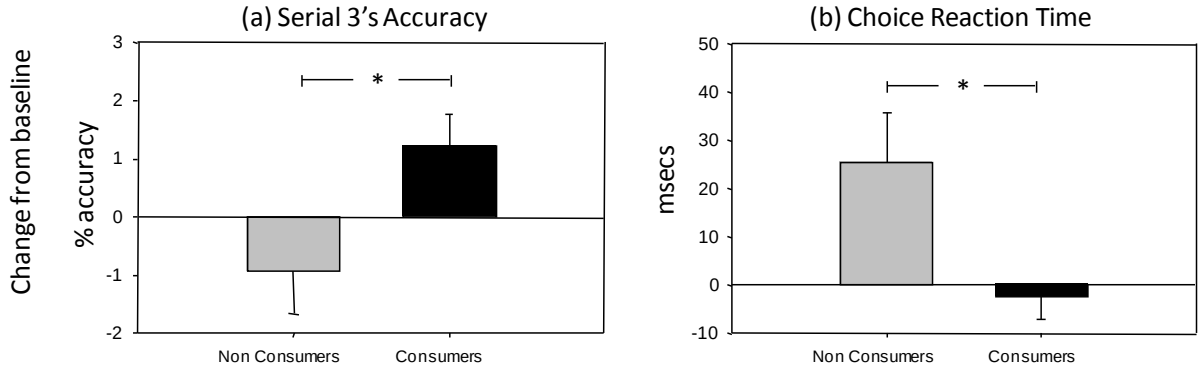


Fig. 4.8. Mean (and SEM) change from baseline scores for cognitive performance. Main effects of consumer status are shown for (a) serial 3's accuracy, (b) choice reaction time (* p<0.05).

4.3.4 Blood pressure and heart rate

4.3.4.1 Systolic blood pressure

Analysis of blood pressure levels revealed a significant main effect of treatment for systolic [$F(3, 66)=4.41$, $p<0.01$]. Planned comparisons demonstrated that following the combination treatment systolic blood pressure was significantly elevated [$t(66)=2.03$, $p<0.05$, $d=-0.57$] as compared to placebo, see fig. 4.9a.

4.3.4.2 Diastolic blood pressure

A significant main effect of treatment was observed for diastolic blood pressure [$F(3, 66)=3.87$, $p<0.05$]. Planned comparisons revealed that diastolic blood pressure was significantly elevated in comparison to placebo following the combination [$t(66)=2.75$, $p<0.01$, $d=-0.76$], caffeine in isolation [$t(66)=3.10$, $p<0.005$, $d=-0.72$] and L-theanine in isolation [$t(66)=2.19$, $p<0.05$, $d=-0.60$], see fig. 4.9b.

4.3.4.3 Heart rate

There were no significant effects of treatment on heart rate.

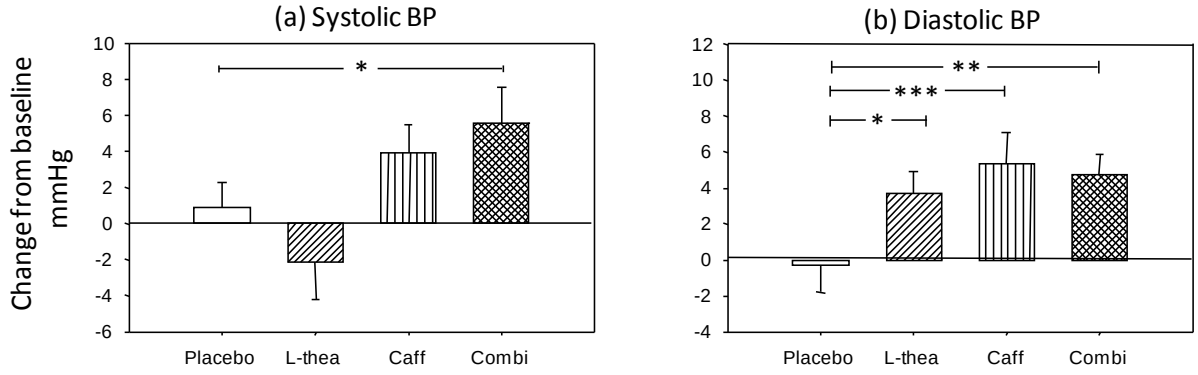


Fig. 4.9. Mean (and SEM) change from baseline blood pressure readings following placebo, 50 mg L-theanine (L-thea), 75 mg caffeine (Caff) and a combination of 50 mg L-theanine and 75 mg caffeine (Combi). Main effects of treatment are shown for (a) systolic and (b) diastolic (* p<0.05, ** p<0.01, *** p<0.005).

4.4 Discussion

The results here demonstrate that, compared to placebo, caffeine administered alone leads to a significant reduction in oxygenated haemoglobin in the pre-frontal cortex during minutes 3-6 and 11-18 of the absorption period and during task performance commencing at 39 minutes post-dose until the end of testing. This effect of caffeine was abolished by combining it with L-theanine, despite no effects of L-theanine in isolation on this measure. Conversely, the expected reduction in deoxygenated haemoglobin during the task period was attenuated following both of the treatments that contained caffeine, as compared to placebo. Following caffeine alone this effect reached significance during

serial subtractions, SRT, RVIP, Stroop and the rest period; following the combination this effect reached significance during serial subtractions, CRT and Stroop. In addition, there was a significant treatment x consumer interaction, whereby the effects on deoxygenated haemoglobin following caffeine alone were predicated on an exaggerated increase in non-habitual consumers throughout the entire testing session.

In relation to the behavioural effects, caffeine significantly reduced reaction time on the CRT task (predominantly in non-habitual consumers) and improved performance accuracy on the Stroop task. It also led to improved subjective ratings of overall mood and alertness (largely due to a reduction in tiredness). The combination treatment was found only to significantly reduce CRT in non-habitual consumers. L-theanine administration also only led to a single effect, manifest as an increase in accuracy of Stroop responses, irrespective of consumer status. Overall and irrespective of treatment habitual consumers were found to be significantly more accurate on the serial threes subtraction task and significantly faster on the CRT task as compared to non-habitual consumers.

In terms of peripheral effects, diastolic blood pressure was significantly elevated following all three active treatments, whereas systolic blood pressure was significantly elevated following the combination treatment only.

In terms of the effects on CBF, in the present study, the expected task-related increase in oxy-Hb was observed following all treatments, for the majority of tasks. However, there was a significant attenuation of this effect following caffeine. Caffeine is a known vasoconstrictor and its effects on cerebral blood flow have been documented previously in imaging studies using higher doses (Chen & Parrish, 2009b; Rack-Gomer et al., 2009) and more recently in a study utilising NIRS that administered the same, comparatively low dose of caffeine as here (Kennedy & Haskell, 2011). However, despite a similar pattern of effects, the previously observed reduction in total levels of haemoglobin following caffeine (Kennedy & Haskell, 2011) failed to reach significance in the current study. Since the total level of haemoglobin is the sum of oxy-Hb and deoxy-

Hb, this is most likely due to a pattern of higher deoxy-Hb and possibly relates to small differences in the levels of caffeine consumed by the non-habitual consumers across the two studies. Of particular interest, the effects of caffeine on oxy-Hb, but not deoxy-Hb were abolished by co-administration with L-theanine. This pattern of effects is consistent with those previously observed on blood pressure by Rogers et al. (2008), showing attenuation of the rise in blood pressure following caffeine in isolation when it was combined with L-theanine. Although higher doses of caffeine and L-theanine were used in that study (250 mg and 200 mg respectively), the ratio of compounds was more comparable to those used here than other studies in this area and the post-treatment assessment took place within a similar time frame (45 minutes post-dose).

The significant increase in deoxy-Hb observed during the task period following caffeine relative to placebo is contrary to the expected increase in oxy-Hb and, or, corresponding decrease in deoxy-Hb generally observed in NIRS assessments during cognitively demanding tasks (Fallgatter & Strik, 1998; Izzetoglu et al., 2004; Izzetoglu et al., 2003; Kennedy, Wightman, et al., 2010; Shibuya-Tayoshi et al., 2007) taken to be indicative of neuronal activation. It is, however not unexpected given the previously observed neurovascular uncoupling following caffeine (Laurienti et al., 2003; Mulderink et al., 2002; Perthen et al., 2008). Previous calibrated (to hypercapnia) BOLD fMRI studies have shown that a high single dose of caffeine (~200 mg+), administered to habitual consumers uncouples the relationship between CBF and local oxygen consumption by reducing blood flow, but increasing oxygen consumption in response to task/stimulation (Chen & Parrish, 2009a; Perthen et al., 2008). This suggests that the increase in deoxy-Hb may be indicative of neural activation. Laurienti et al. (2002) also showed that the effects of 250 mg caffeine administration on fMRI BOLD were correlated to dietary caffeine use with high consumers (mean 648 mg/day), tending to show increased activation and lower consumers (mean 41 mg/day) showing decreased activation. The differential effect of consumer status on deoxy-Hb in the current study reflecting a significant increase in deoxy-Hb across tasks following caffeine in non-habitual consumers only, suggests that the disparity of effects of caffeine on BOLD activation are the result of

varying deoxy-Hb rather than oxy-Hb response. Given the lack of interaction effect on oxy-Hb in the current study, this may point to a specific tolerance to the effects on deoxy-Hb in habitual caffeine consumers. As the effects on deoxy-Hb suggest increased neural activation in non-consumers, this would indicate an upregulation of A_1 receptors (Laurienti et al., 2003), as has previously been demonstrated (Johansson et al., 1993). Interestingly no differential effects on deoxy-Hb as a function of consumer status were observed following the combination of caffeine and L-theanine, with both groups showing an increase.

Turning to the behavioural findings, the relative lack of effects following the combination of caffeine and L-theanine in the current study is contrary to previous findings. Combinations of caffeine and L-theanine have previously been shown to improve performance on SRT and RVIP tasks and improve subjective alertness, tiredness, mental fatigue and headache ratings (Einother et al., 2010; Haskell et al., 2008b; Kelly et al., 2008; Owen et al., 2008); however, in the present study no such improvements were observed. It is somewhat surprising given that the combination showed a similar but lesser modulation of deoxy-Hb to caffeine, but did not decrease oxy-Hb (in fact this was increased during the final two epochs of the absorption period). The lack of behavioural effects may be due to the lower doses used here in addition to the ratios of caffeine and L-theanine administered (in the aforementioned studies the combinations comprised ratios of caffeine and L-theanine, that were more in favour of L-theanine). However, despite the different ratios and lower doses used in the current study, the improvements seen as a result of the combination treatment on choice reaction time are consistent with those observed previously (Haskell et al., 2008b; Owen et al., 2008). These effects were, however, dependent upon a participant's level of caffeine consumption, as reaction time was only improved in non-habitual consumers. An effect of consumer status was also observed following the caffeine-in-isolation treatment, as an improvement in choice reaction time was similarly, only observed in non-consumers. These effects of consumer status were largely due to there being very little difference in reaction times across treatments for the habitual consumer group, with one explanation being that the doses

used in the present study were too low to elicit an effect in habitual consumers. However, this justification seems unlikely, as previous studies have demonstrated significant improvements in reaction time of this and lower doses in both habitual consumers and habitual non-consumers (Haskell et al., 2005; Richardson, Rogers, Elliman, & Odell, 1995; Smit & Rogers, 2000). An alternative explanation is that habitual consumers were the subject of ceiling effects, indicated by an almost identical level of performance on this task following all treatments. This is supported by an overall consumer status effect showing habitual consumers to be significantly faster on the CRT task as compared to non-habitual consumers, irrespective of treatment; as well as being more accurate on the serial 3s subtraction task. The demonstration of an improvement in CRT in non-habitual consumers (in the absence of significant differences between consumer groups at baseline), in addition to significant differences in favour of habitual consumers, would seem to indicate that these were net effects of treatment (Haskell et al., 2005; Hewlett & Smith, 2006; Smith, Christopher, & Sutherland, 2013) and not those of a reversal of withdrawal (Rogers et al., 2003). Indeed, better performance on the serial 3s subtraction task for habitual consumers has previously been demonstrated (Haskell et al., 2005). However, it is not clear whether differing responses of habitual and non-habitual consumers of caffeine are the consequence of chronic caffeine consumption or are related to underlying differences. Caffeine in isolation also improved alertness and overall mood, both of which have been previously reported (Childs & de Wit, 2006; Haskell et al., 2005; Rogers et al., 2008) and improved accuracy on the Stroop task, a finding not unexpected considering caffeine's known, positive effects on attention (Haskell et al., 2005). L-theanine in isolation was also found to improve accuracy on the Stroop task. In contrast to those following caffeine, this finding could be described as unexpected, as although no other studies to date have assessed the effects of L-theanine alone on the performance of this task, previous research has only reported decrements or no effects on performance when L-theanine is administered in isolation (Gomez-Ramirez et al., 2007; Gomez-Ramirez et al., 2009; Haskell et al., 2008b; Kelly et al., 2008; Owen et al., 2008). Once again, the dose used here may provide an explanation for this, as the majority of

studies to date have administered doses of L-theanine much higher than used in the current study.

In terms of the peripheral effects, the combination treatment was found to increase systolic blood pressure and all three active treatments increased diastolic blood pressure. The ability of caffeine to raise blood pressure has been documented previously (James, 2004; Rogers et al., 2008; Umemura et al., 2006) and the rise seen in the present study is as expected. In contrast, the increase seen following L-theanine is perhaps counter-intuitive based on L-theanine's purported historic use as a relaxing agent (Heese et al., 2009) its ability to reduce induced stress responses (Kimura et al., 2007) and evidence that it is capable of reducing blood pressure in rats (Yokogoshi et al., 1995). It should be noted that this effect in rats was only observed following very high doses (1500 and 2000mg/kg), in spontaneously hypertensive rats, not normotensive Wistar Kyoto rats. An effect of L-theanine in isolation on blood pressure in humans has only been demonstrated in 'high-responders' as determined by placebo blood pressure response to a mental task (Yoto, Motoki, Murao, & Yokogoshi, 2012). In terms of the combination, this treatment was found to significantly increase both systolic and diastolic blood pressure. This is in contrast to the findings of Rogers et al., (2008), who found that when caffeine was combined with L-theanine it was able to attenuate the rise in blood pressure seen following caffeine in isolation. However, Geisbrecht et al. (2010) found that 40 mg caffeine combined with 97 mg L-theanine led to significant increases in systolic blood pressure with a trend towards the same for diastolic. These contrasting effects of caffeine/L-theanine combinations on blood pressure may be indicative of differential effects of different doses on this parameter. Other methodological differences should also be noted; Rogers et al. (2008) did not restrict intake of caffeine/L-theanine whereas in the current study and that of Geisbrecht et al. (2010) caffeine and theanine intake was prohibited for 12 hours prior to study visits. The tasks employed by Rogers et al. (2008) could also be said to be of a lower complexity than those administered in the latter studies prior to blood pressure recording.

The present study has demonstrated that caffeine and L-theanine at doses equivalent to 1 to 2 cups of tea are capable of modulating cerebral haemodynamics, cognitive performance, mood and autonomic measures. When combined with 75 mg caffeine, 50 mg L-theanine antagonised the effects on oxy-Hb observed during cognitive tasks following 75 mg caffeine in isolation. As the attenuation of the reduction in oxy-Hb continued until the end of testing, future studies should aim to determine the duration of these effects on caffeine by L-theanine by extending the post dose testing period. This study also provides partial replication of a previous study showing modulation of the CBF effects of 75mg caffeine as a function of habitual caffeine consumption (Kennedy & Haskell, 2011). In the current study, the combination of caffeine and L-theanine also resulted in a reduction in the effectiveness of caffeine to modulate behaviour, but it should be noted that when consumed in the form of tea there are many other compounds present with the potential to interact with the two compounds explored here. Once again the technique of NIRS has demonstrated its ability to identify cerebral oxygenation changes following administration of a nutritional intervention, this time one known for its ability to reduce cerebral blood flow. However, it has also demonstrated this it is sensitive enough to detect an attenuation of these parameters when a second intervention is introduced.

Chapter 5: An assessment of energy expenditure and cerebral haemodynamics during cognitive performance, following two doses of caffeine.

5.1 Introduction

Caffeine's impact on metabolism is an area that has received attention in the literature in both healthy and obese populations due to its thermogenic effects. Acheson et al. (1980) observed that caffeine (in the form of caffeinated coffee) significantly increased metabolic rate in both normal weight (8 mg/kg administered) and obese participants (4 mg/kg administered). Similarly, Dulloo et al. (1989) demonstrated an increase in resting metabolic rate in both lean participants and those who were categorised as being pre-disposed to obesity and were previously overweight, following a 100 mg dose of caffeine. In further studies of normal weight participants, an increase in energy expenditure has been demonstrated at rest following 100 mg caffeine (Astrup et al., 1990; Hollands et al., 1981), 400 mg caffeine (Astrup et al., 1990) and a larger dose of 10 mg/kg caffeine (Acheson et al., 2004), with increased fat oxidation demonstrated following 8 mg/kg (Acheson et al., 1980) and 10 mg/kg (Acheson et al., 2004). Research has also extended to exercise models due to caffeine's purported ergogenic effects and the potential for caffeine to enhance exercise performance. One of the first studies to demonstrate the beneficial effects of caffeine during exercise was research conducted by Costill et al. (1978). They observed that coffee containing 330 mg caffeine (as compared to decaffeinated coffee), when administered 60 minutes prior to exercise (bicycle ergometer at 80 % of Vo2 max), not only increased the rate of lipid metabolism and significantly increased fat oxidation, but also significantly increased time to exhaustion. Although the findings in terms of increased time to exhaustion have been demonstrated since (Greer, Friars, & Graham, 2000; Pasman, Vanbaak, Jeukendrup, & Dehaan, 1995; Simmonds, Minahan, & Sabapathy, 2010; Spriet et al., 1992), the findings in relation to substrate metabolism have been equivocal (Graham, Battram, Dela, El-Sohemy, & Thong, 2008). Animal research has shown that the means by which caffeine is thought to

increase time to fatigue is, similar to caffeine's cognitive and haemodynamic effects, via antagonism of adenosine receptors (Davis et al., 2003).

Indirect calorimetry is a method of determining energy expenditure, and carbohydrate and fat oxidation through the measurement of inhaled and exhaled gas. The majority of research that has used ICa to assess the impact of nutritional interventions on metabolism has been conducted using resting or exercise protocols. To date there have been three studies that have used ICa to determine the metabolic impact of cognitive task performance (Kennedy et al., 2016; Seematter et al., 2000; Troubat, Fargeas-Gluck, Tulppo, & Dugue, 2009), only one of which has been in the presence of a nutritional intervention (Kennedy et al., 2016). Seematter et al. (2000) demonstrated that performance of cognitive tasks (arithmetic tasks and Stroop) led to an increase in overall energy expenditure in both lean and obese women. During performance of an hour long game of chess, Troubat et al. (2009) did not observe an effect on energy expenditure; however, there were changes in substrate metabolism. An initial increase in carbohydrate oxidation (with a corresponding reduction in fat oxidation, which failed to reach significance), followed by a significant increase in fat oxidation, with a significant decrease in carbohydrate oxidation during the middle and end of the task was observed. This was taken as an indication of switching substrate oxidation as a function of the demands of the task over time. A study by Kennedy et al. (2016) that looked at the CBF (using NIRS) and metabolic effects of performing cognitive tasks of differing difficulty following the administration of multivitamins and minerals, found that fat oxidation and energy expenditure were significantly increased during cognitive task performance. This effect was observed following acute and (in the case of energy expenditure only) chronic (56 days) supplementation with micronutrients. Cerebral blood flow was also increased during task performance following acute supplementation.

As discussed in chapter 4, the behavioural effects of caffeine have been well documented, with the most consistent effects being increased ratings of alertness as well as improvements in measures of reaction time and vigilance (Haskell et al., 2008b;

Haskell et al., 2005; Quinlan et al., 2000; Rogers et al., 2008). Caffeine's ability to restrict cerebral blood flow is also well established and has been demonstrated via neuroimaging methodologies (Chen & Parrish, 2009a; Laurienti et al., 2003; Mathew & Wilson, 1991; Rack-Gomer et al., 2009) including NIRS (Kennedy & Haskell, 2011). The findings of chapter 4 contributed to this area through the demonstration that NIRS is sensitive enough to detect changes in blood oxygenation during cognitive tasks following the administration of 75 mg caffeine. The evidence from ICa studies suggests that cognitive task performance leads to increases in energy expenditure and may also modulate fat and (to some extent) carbohydrate oxidation during certain tasks. In light of caffeine's influence on metabolism and taking into consideration its cerebro-vascular and cognitive effects, it would be interesting to conduct parallel assessments of both metabolic rate (measured via ICa), and the delivery of metabolic substrates/oxygen extraction (as measured by NIRS).

The present study therefore intended to build on the findings of chapter 4 in relation to cerebral oxygenation by introducing a measure of metabolism during task performance in the presence of caffeine. The completion of cognitive tasks of varying difficulty was intended to delineate any changes in metabolic demand and oxygen delivery/utilisation. It was anticipated that an increase in task demand would be reflected by an increase in energy expenditure and a concomitant increase in blood flow and oxygen extraction, with caffeine potentially interacting with both measures as a result. This double-blind, placebo-controlled, balanced, crossover study aimed to assess the impact of two doses of caffeine on cerebral haemodynamics and whole-body metabolism whilst simultaneously performing of a range of cognitive tasks that differed in their level of demand. The second aim of the study was to explore the impact of tasks of different levels of subjective difficulty on physiological measures (CBF and metabolism), irrespective of the treatment administered.

5.2 Method

5.2.1 Participants

Twenty-four healthy young participants (13 males, 11 females) between the ages of 18 and 35 (mean age 23.5, SD 3.8; BMI 23.0, SD 2.6) were recruited. The study was approved by Northumbria University's School of Psychology and Sport Sciences' ethics committee and conducted in accordance with the Declaration of Helsinki. Prior to participation, volunteers were required to sign an informed consent form and complete a self-report caffeine consumption questionnaire (appendix C) that assessed their daily level of caffeine intake. Volunteers were recruited to take part in the study if they were 'habitual consumers' (those who drank tea and/or coffee and consumed more than 150 mg caffeine, but no more than 600 mg per day). The decision to only recruit habitual consumers was taken to remove any impact of consumption status, as identified in chapter 4. From the self-report caffeine consumption questionnaire, participants reported drinking between 156 mg to 555 mg caffeine per day (mean 327.0, SD 90.2). A general health screen (appendix B) informed volunteers that they would not be eligible to take part if they had a history of neurological, vascular or psychiatric illness or a history or current diagnosis of drug or alcohol abuse. They would also not be eligible if they had a current diagnosis of depression or anxiety, anaemia, high blood pressure, a heart or respiratory disorder, type 1 diabetes, phenylketonuria, a history of head trauma, migraines, learning difficulties, dyslexia or ADHD. All participants reported that they were in good health, had normal or corrected-to-normal vision and had no known allergies to the treatment ingredients. Additionally, they were not currently taking any dietary supplements or medication (including the contraceptive pill), were not colour-blind, did not smoke and in the case of female volunteers, were not pregnant or seeking to become pregnant.

5.2.2 Design and treatment

A randomised, double-blind, counter-balanced, within subjects, placebo-controlled design was utilised. Participants attended 3 study visits and at each received 1 of the following treatments: 75 mg caffeine (pharmaceutical grade caffeine powder, Blackburn Distributions Ltd); 150 mg caffeine or placebo. Each treatment was administered in the

form of a capsule in order to mask any taste differences and ensure participants remained blind to the treatment they had received. The order in which participants received each treatment was determined by Latin square and random allocation to treatment order.

5.2.3 Salivary caffeine levels

Saliva samples were obtained using salivettes (Sarstedt Ltd). One sample was taken upon arrival and one immediately following the post-dose assessment. This was to ensure overnight caffeine abstinence and to confirm caffeine absorption following treatment. Once taken, samples were frozen at -80 ºC. The samples were then thawed and the caffeine levels in the saliva samples were measured using an Emit® Caffeine Assay (Dade Behring Ltd).

5.2.4 Physiological, cognitive and mood measure

5.2.4.1 Near infrared spectroscopy measurements

Please see chapter 2 for a full description of the NIRS method used, which is identical to that used in the present chapter.

5.2.4.2 Cognitive and mood measures

Please see chapters 2 and 3 for a description of COMPASS and an explanation of tasks and mood assessments listed here but not described in full below. The tasks were chosen based on their ability to activate the pre-frontal cortex (Cohen et al., 1997; Drummond et al., 1999; Lawrence et al., 2002) or their known sensitivity to caffeine (Haskell et al., 2008b; Kennedy & Haskell, 2011). In an attempt to avoid any effects of task order in relation to metabolic parameters, the order in which the tasks were completed at baseline and post-dose were determined by a counterbalancing Latin square and randomly allocated for each participant. This order was consistent at each study visit and for baseline and post-dose sessions. All baseline tasks were 2 minutes and all post-dose tasks were 5 minutes in duration, with a 2-minute rest period observed after each post-dose task in order to limit any overlapping metabolic effects of the previous task.

5.2.4.2.1 Serial 3s:

Please see chapter 2 for a description of this task. This task was scored for percentage accuracy and percentage errors.

5.2.4.2.2 Serial 7s:

Please see chapter 2 for a description of this task. This task was scored for percentage accuracy and percentage errors.

5.2.4.2.3 Serial 17s:

Please see chapter 3 for a description of this task. This task was scored for percentage accuracy and percentage errors.

5.2.4.2.4 RVIP:

Please see chapter 2 for a description of this task. This task was scored for percentage of target strings correctly detected, average reaction time for correct detections, and number of false alarms.

5.2.4.2.5 3-back:

Please see chapter 2 for a description of this task. This task was scored for percentage of correct responses and reaction time.

5.2.4.2.6 Key tapping control:

This task was included as a control to account for physical activity required during task performance in relation to metabolism and CBF. It was therefore somatically matched to the task that required the most physical movement (in this instance, serial 3s). It also provided a comparator for the tasks in terms of subjective difficulty. To the sound of a metronome (set at 84 beats per minute; derived from the average frequency of key strokes during the serial 3s task) participants are instructed to alternately press the ‘C’ key and the ‘N’ key on the keyboard using their left and right forefingers respectively. This task is not scored.

5.2.4.2.7 Subjective mental fatigue visual analogue scale:

Following completion of each task at post-dose, participants rated their level of mental fatigue. Please see chapter 2 for a description of this task.

5.2.4.2.8 Subjective difficulty visual analogue scale:

Following completion of each task at post-dose, participants rated the difficulty of each task by placing an 'x' on a 100 mm line with the end points labelled 'not at all' (left hand end) and 'extremely' (right hand end).

5.2.4.2.9 Caffeine research visual analogue scales:

Following completion of all tasks at the baseline and post-dose sessions, participants completed these scales. Please see Chapter 4 for a description of this task.

5.2.4.2.10 Subjective energy measures:

Following completion of all tasks at the baseline and post dose sessions, scales which assessed the participants' self-ratings of 'concentration', 'mental stamina' and 'physical stamina' were completed. Participants were asked to rate how much they matched their current state by placing an 'x' on a 100 mm line, with 'very low' labelled at the left-hand end and 'very high' labelled at the right-hand end. Participants' self-rated levels of being 'mentally tired' and 'physically tired' were then completed in the same way, with the scales labelled 'not at all' and 'extremely'.

5.2.4.3 Indirect calorimetry

During completion of cognitive tasks, pulmonary (minute) ventilation (VE), oxygen uptake (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER) were determined from breath-by-breath expired gases, measured using indirect open circuit calorimetry (Cortex Metalyzer 3B, Cranlea, UK). Participants breathed as normal into a mask that covered the nose and mouth, which was connected using falconia tubing to the Metalyzer. This data was then used to determine measures of energy expenditure (kcal/min), carbohydrate oxidation (g/min) and fat oxidation (g/min), using standard formulae, as described by Frayn (1983).

5.2.4.4 Blood pressure and heart rate

Please see chapter 3 for details of equipment used. Readings were taken from the upper left arm following 5 minutes seated rest at each visit upon arrival and following post-dose completion of the cognitive tasks.

5.2.5 Procedure

Participants were required to attend the laboratory on 4 separate occasions. The first visit was a screening session where participants were informed about the nature of the study, its requirements and its restrictions. Informed consent was obtained and their eligibility to participate was confirmed. Habitual caffeine intake and source were assessed via questionnaire and familiarisation with the tasks to be administered on the study days was conducted. The remaining 3 study visits were identical to each other, with the exception of the treatment administered. On each day, participants attended the lab at 9.30 am following an overnight 12-hour fast where they were permitted to only drink water. Upon arrival, salivary caffeine levels were taken to ensure caffeine abstinence and following 5 minutes of seated rest heart rate and blood pressure readings were taken. Following this, the ICa face mask and the NIRS headband were fitted. At this point NIRS recording began. Participants then made a baseline completion of the cognitive tasks, the caffeine visual analogue scales and the subjective energy measures. The gas turbine was then inserted into the face mask and recording of respiratory gas analysis began. Participants were required to sit quietly for a 10 minute NIRS and gas analysis resting baseline period (during which time participants watched a non-stimulating home improvement DVD). Gas analysis was then stopped, the turbine was removed and participants were required to take their treatment for the day. Following a 30-minute absorption period (during which time NIRS recording continued whilst participants watched the DVD), the gas turbine was re-inserted into the face mask and post-dose resting gas analysis readings were taken for a further 5 minutes whilst participants continued to watch the DVD. Participants then completed a second set of the cognitive tasks. Following each of the post-dose tasks participants were required to rate their level

of mental fatigue and the subjective difficulty of each task. A 2-minute resting period was also completed between each task, to minimise any carry-over effects on CBF from the previous task. Following the cognitive tasks, the caffeine visual analogue scales and the subjective energy measures were completed for a second time, blood pressure and heart rate were measured and a final saliva sample was taken and used to confirm caffeine absorption (see fig 5.1 for more details of procedure and task duration). Participants returned for their next study visit following (at least) a 48-hour washout period.

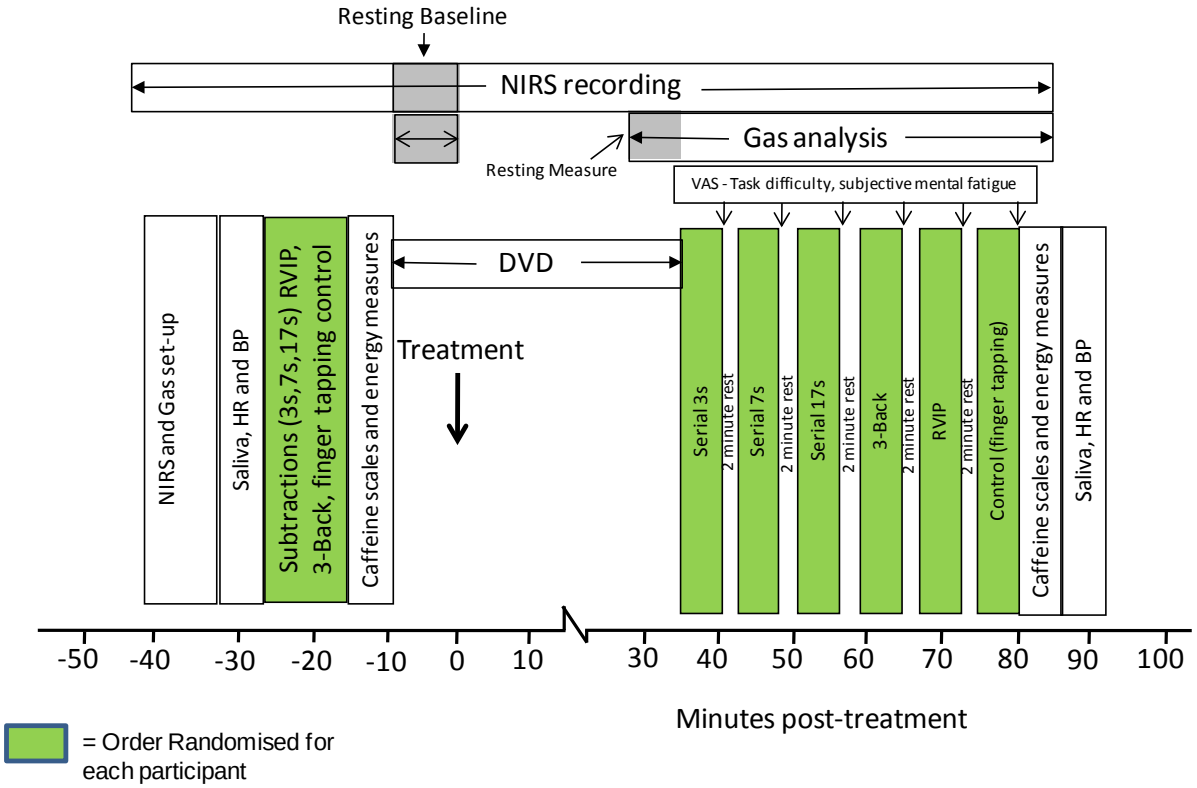


Fig. 5.1. Timeline representing flow of study day.

5.2.6 Statistics

Two paired sample t-tests were utilised to analyse baseline and post-dose salivary caffeine levels to confirm caffeine absorption. To determine if any differences existed in salivary caffeine levels following the 75 mg and 150 mg caffeine treatments, 'change from baseline' data were analysed by paired samples t-test.

Prior to the primary NIRS analysis a within subjects ANOVA was carried out with left/right optode included as a factor to examine any treatment related hemispheric differences in response. As there were no interpretable interactions involving this factor the data from the two channels were averaged for the analysis.

For the primary NIRS analysis, the question under investigation was how the two doses of caffeine would modulate the haemodynamic response over time as compared to placebo, irrespective of which task was being completed at that time. Data for oxy-Hb, deoxy-Hb and total-Hb was averaged across 5 minute epochs during the absorption and task period and baseline adjusted to the last 5 minutes of the post-task resting pre-treatment period. Data was then analysed by 2-way repeated measures ANOVA (treatment (75 mg caffeine, 150 mg caffeine or placebo) X epoch (13 x 5 minute epochs)). Significant treatment related interactions were then described, with reference to *a priori* planned comparisons, where each active treatment was compared to placebo at each epoch utilising t tests calculated with the Mean Squares Error from the ANOVA (Keppel, 1991). In order to reduce the potential for Type I errors only those planned comparisons associated with a statistically significant difference on the initial ANOVA are reported. In addition, only those instances where a consistent pattern of significant differences are maintained across epochs are identified as reportable significant effects.

In order to identify the specific effects of task performance, a further analysis was conducted whereby the task period was analysed alone by 2-way repeated measures ANOVA (treatment (as above) X task (serial 3s, serial 7s, serial 17s, 3-back, RVIP, control). Planned comparisons were conducted as per primary analysis, documented above.

Secondary analysis was aimed at assessing in more detail the effect of treatment on tasks through the use of shorter-duration epochs. In order to identify the course of treatment-related haemodynamic effects over the first and second half of each task, data was baseline adjusted to the last 5 minutes of the post-task resting pre-treatment period. Analysis of the task period was conducted by two-way repeated measures ANOVA

(treatment (as per primary analysis) X (12 x 2.5 minute epochs)). Planned comparisons for secondary analysis were conducted as per primary analysis, documented above.

The primary analysis of indirect calorimetry data aimed to identify treatment-related effects of individual task performance. Measures of energy expenditure, carbohydrate oxidation and fat oxidation were calculated and averaged across each 5-minute task period. Raw data was then analysed by 2-way repeated measures ANOVA (treatment (75 mg caffeine, 150 mg caffeine or placebo) X task (as per NIRS assessment)). The rest period was analysed alone by 1-way repeated measures ANOVA. Significant treatment related interactions were described with reference to *a priori* planned comparisons as per NIRS assessment.

The secondary aim of the study was to explore the effects of task performance (irrespective of treatment) on measures of metabolism and CBF. Therefore, where a significant main effect of task was observed during the 2-way repeated measures ANOVA (treatment X task) from the primary analysis for NIRS and ICa measures, pairwise comparisons (partial Bonferroni corrections) were conducted on each task as compared to the control.

To assess the possibility of any on-day differences in cognitive performance, ICa, mood, blood pressure and heart rate pre-treatment, repeated measures ANOVAs were conducted on baseline data. Any significant differences were further explored with Bonferroni corrected pairwise comparisons.

Cognitive performance, subjective mood, heart rate and blood pressure data were analysed as 'change from baseline' by repeated measures ANOVA (apart from post-individual-task subjective measures of difficulty and mental fatigue, where raw data was used). Significant treatment related effects were described with reference to *a priori* planned comparisons (as above) where each active treatment was compared to placebo.

5.3 Results

5.3.1 Salivary caffeine levels

Prior to analysis, baseline salivary caffeine levels were examined and it was confirmed that they were in accordance with levels of caffeine that would reflect overnight caffeine abstinence. Mean baseline values were 0.65 µg/ml [levels below 1 µg/ml have previously been reported for overnight caffeine abstinence (Evans & Griffiths, 1999)]. Following caffeinated treatment, salivary caffeine levels were 1.42 µg/ml (75mg caffeine) and 2.92 µg/ml (150 mg caffeine). Analysis of the results confirmed that in comparison to baseline levels, salivary caffeine was significantly higher following 75 mg caffeine [$t(23) = -4.05$, $p<0.001$] and 150 mg caffeine [$t(23) = -14.69$, $p<0.001$], (see fig. 5.2). There was also a significant difference between salivary caffeine levels post-treatment, whereby the 150 mg dose elicited a significantly greater rise in salivary caffeine levels than the 75 mg dose [$t(23) = -6.08$, $p<0.001$].

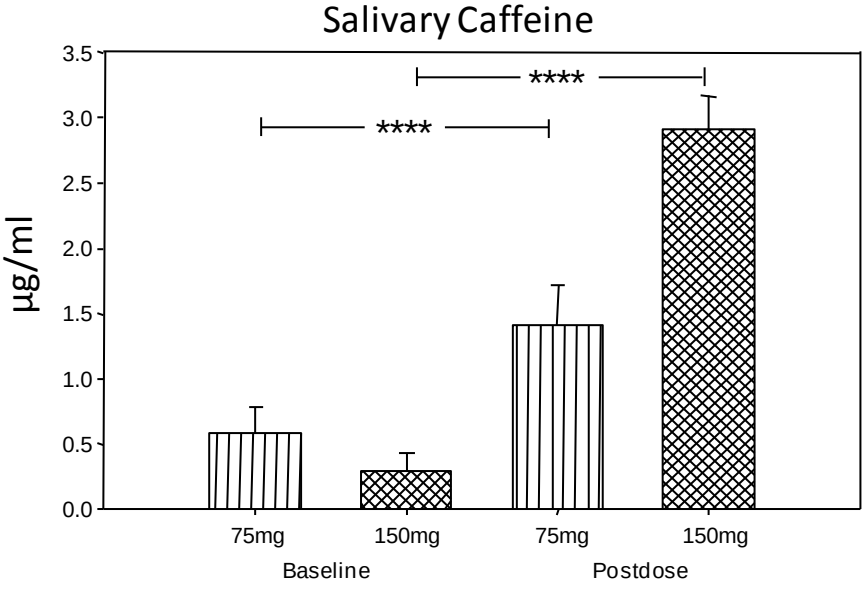


Fig. 5.2. Mean (and SEM) salivary caffeine values at baseline and post-dose following 75 mg caffeine and 150 mg caffeine. Paired sample t-test significance levels are shown (**** p<0.001).

5.3.2 Treatment related effects

5.3.2.1 Near infrared spectroscopy; primary analysis

Effects of treatment on cerebral blood flow over time, irrespective of task performed.

5.3.2.1.1 Oxygenated haemoglobin

There were no treatment related differences in oxy-Hb over time, see fig. 5.3a.

5.3.2.1.2 Deoxygenated haemoglobin

There were no treatment related differences in deoxy-Hb over time, see fig. 5.3b.

5.3.2.1.3 Total haemoglobin

A significant interaction effect (treatment x epoch) was observed for total-Hb [$F(24, 552)=1.65$, $p<0.05$]. Planned comparisons revealed that total-Hb was significantly increased during minutes 6-10 [$t(552)=2.238$, $p<0.05$, $d=0.52$], 16-20 [$t(552)=2.130$, $p<0.05$, $d=0.42$] and 26-30 [$t(552)=2.331$, $p<0.05$, $d=0.40$] of the absorption period following 75 mg caffeine as compared to placebo, see fig. 5.4.

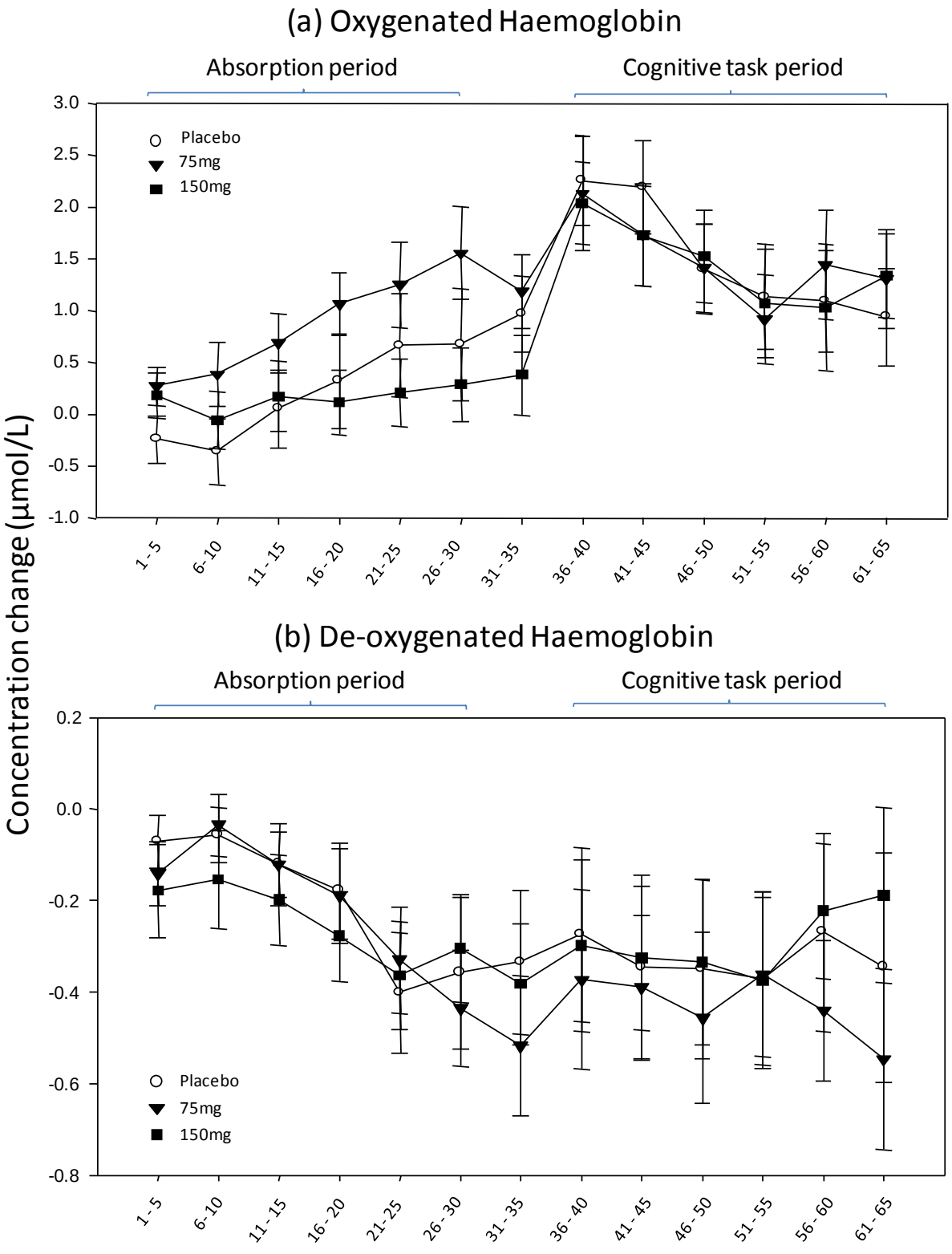


Fig. 5.3. Concentration changes of oxy-Hb (a) and deoxy-Hb (b) represented in 5 minute epochs during absorption and cognitive task period over time (irrespective of task performed) following placebo, 75 mg caffeine and 150 mg caffeine. Means and SEM are presented as change from pre-treatment, resting baseline.

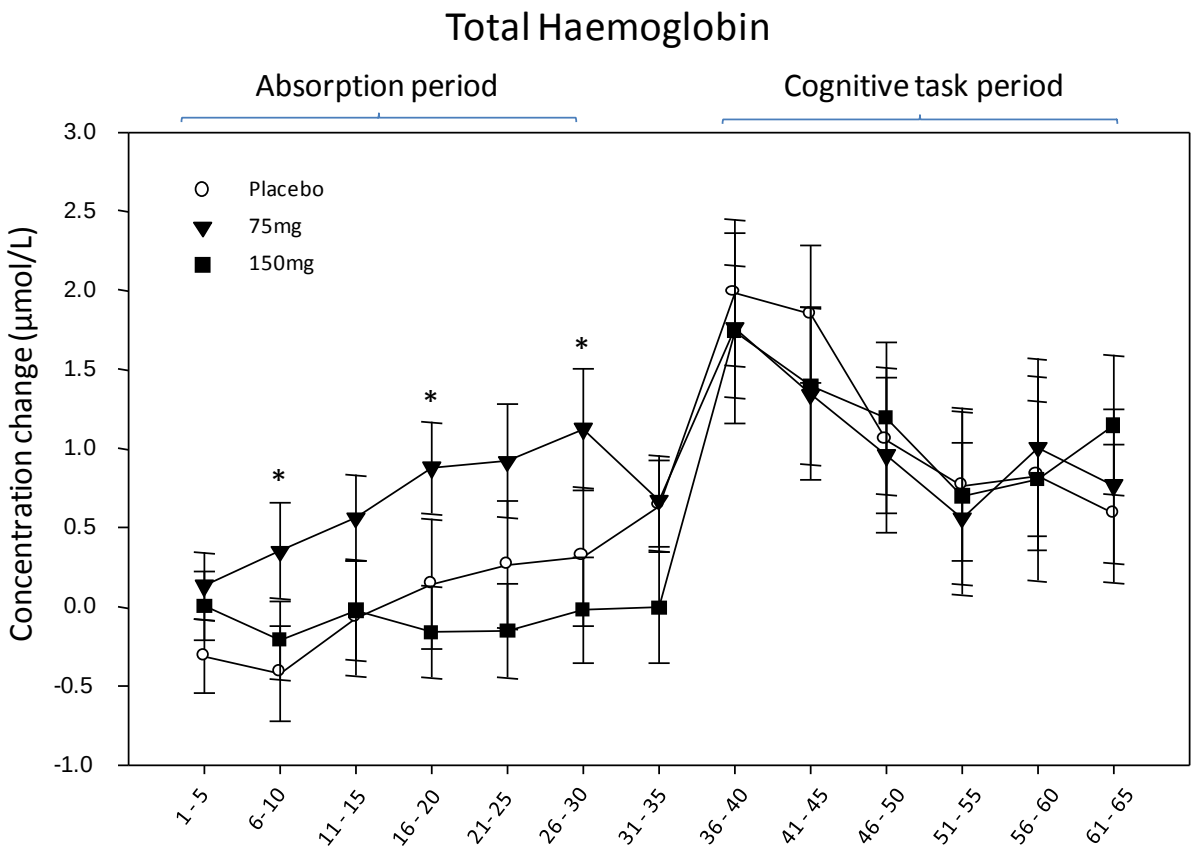


Fig. 5.4. Concentration change of total-Hb represented in 5 minute epochs during absorption and cognitive task period over time (irrespective of task performed) following placebo, 75 mg caffeine and 150 mg caffeine. Means and SEM are presented as change from pre-treatment, resting baseline. Treatment x epoch interaction effects are shown. Significance is compared to placebo (t-tests calculated with the Mean Squares Error from the ANOVA) (* $p<0.05$).

5.3.2.1.4 Further primary analysis

Effects of treatment on cerebral blood flow during performance of individual tasks.

5.3.2.1.4.1 Oxygenated haemoglobin

There were no treatment related differences specific to individual tasks for oxy-Hb.

5.3.2.1.4.2 Deoxygenated haemoglobin

There were no treatment related differences specific to individual tasks for deoxy-Hb.

5.3.2.1.4.3 Total haemoglobin

There were no treatment related differences specific to individual tasks for total-Hb.

5.3.2.2 Near infrared spectroscopy; secondary analysis

Effects of treatment on cerebral blood flow during first and second half of individual task performance.

5.3.2.2.1 Oxygenated haemoglobin

There were no treatment related differences in oxy-Hb specific to task performance.

5.3.2.2.2 Deoxygenated haemoglobin

There were no treatment related differences in deoxy-Hb specific to task performance.

5.3.2.2.3 Total haemoglobin

There were no treatment related differences in total-Hb specific to task performance.

5.3.2.3 Indirect calorimetry

Effects of treatment on metabolism during performance of individual tasks and during rest period.

5.3.2.3.1 Baseline scores

There were no significant on-day differences in measures of energy expenditure, carbohydrate oxidation or fat oxidation prior to treatment. Due to data capture errors, only data from 13 participants is included the primary analysis.

5.3.2.3.2 Energy expenditure

There was a significant main effect of treatment on energy expenditure during task performance [$F(2, 120)=3.803$, $p<0.05$]. Planned comparisons revealed that energy expenditure was significantly higher following 75 mg [$t(120)=2.48$, $p<0.05$, $d=0.40$] and 150 mg caffeine [$t(120)=3.81$, $p<0.001$ $d=0.59$] as compared to placebo, see fig. 5.5a. There were no treatment related effects on energy expenditure during the rest period.

5.3.2.3.3 Carbohydrate oxidation

There was a significant main effect of treatment on carbohydrate oxidation during task performance [$F(2, 120)=8.099$, $p<0.005$]. Planned comparisons revealed that carbohydrate oxidation was significantly higher following 75 mg [$t(120)=3.89$, $p<0.001$, $d=0.77$] and 150mg caffeine [$t(120)=5.90$, $p<0.001$, $d=0.95$], as compared to placebo, see fig. 5.5b. There was a significant main effect of treatment on carbohydrate oxidation during the rest period [$F(2, 24)=11.33$, $p<0.001$]. Planned comparisons revealed that during the rest period, carbohydrate oxidation was significantly higher following 75 mg [$t(24)=4.13$, $p<0.001$, $d=1.19$] and 150 mg caffeine [$t(24)=4.40$, $p<0.001$, $d=1.12$], as compared to placebo, see fig. 5.6a.

5.3.2.3.4 Fat oxidation

There were no treatment related effects on fat oxidation during task performance, see fig. 5.5c. There was a significant main effect of treatment on fat oxidation during the rest period [$F(2, 24)=7.58$, $p<0.005$]. Planned comparisons revealed that during the rest period, fat oxidation was significantly lower following 75 mg [$t(24)=3.62$, $p<0.005$, $d=-.93$] and 150 mg caffeine [$t(24)=3.06$, $p<0.01$, $d=-0.73$], as compared to placebo, see fig. 5.6b.

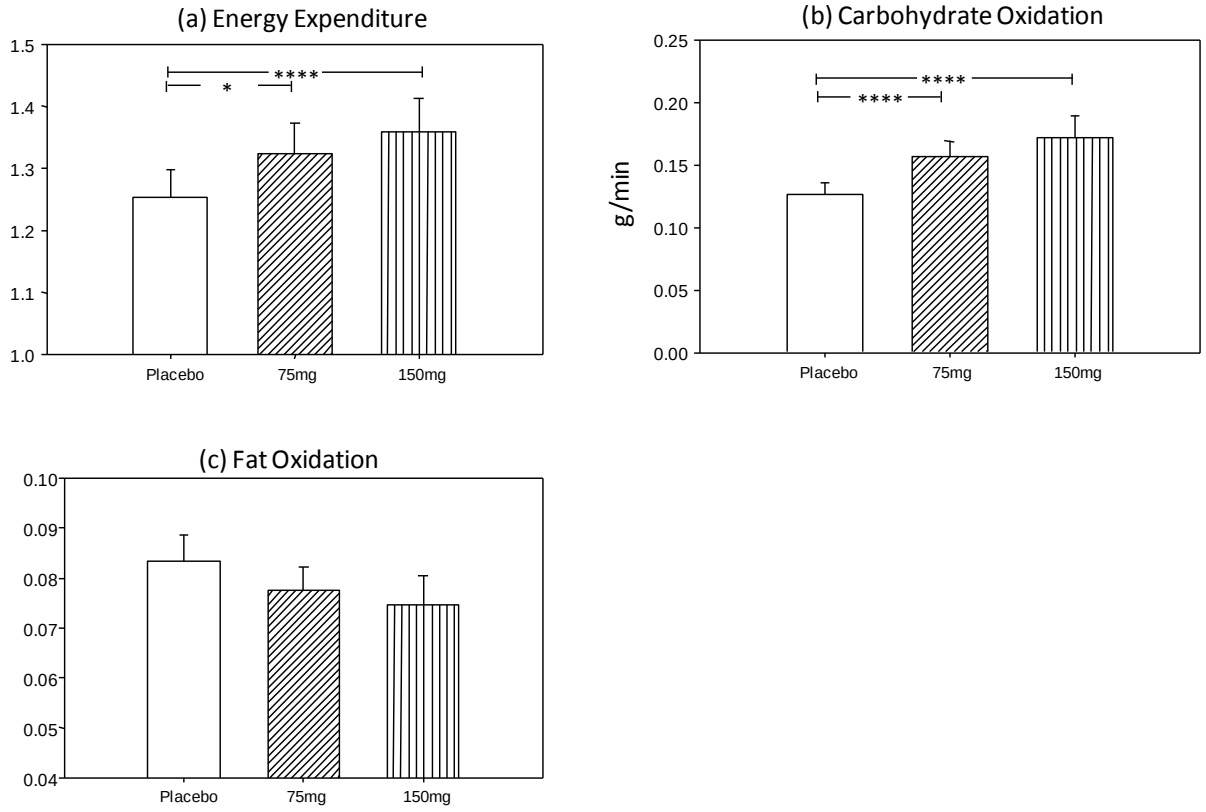


Fig. 5.5. Raw mean (and SEM) values for (a) energy expenditure, (b) carbohydrate oxidation and (c) fat oxidation following placebo, 75 mg caffeine and 150 mg caffeine. Main effects of treatment are shown, significance is compared to the placebo (t-tests calculated with the Mean Squares Error from the ANOVA) (* p<0.05, **** p<0.001).

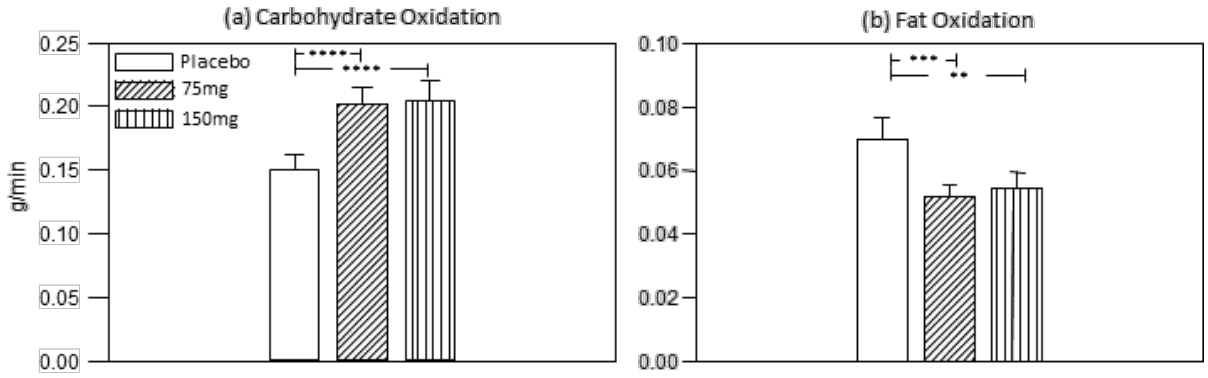


Fig. 5.6. Raw mean (and SEM) values for (a) carbohydrate oxidation and (b) fat oxidation during the post-treatment rest, following placebo, 75 mg caffeine and 150 mg caffeine. Main effects of treatment are shown, significance is compared to the placebo (t-tests calculated with the Mean Squares Error from the ANOVA) (** p<0.01, *** *p*<0.005, **** p<0.001).

5.3.2.4 Cognitive performance and mood

Effects of treatment on measures of cognitive performance and mood.

5.3.2.4.1 Baseline scores

There were no significant on-day differences in cognitive performance, mood or autonomic measures prior to treatment. Due to an oversight when programming COMPASS, the alertness scale was absent from the configuration for the post-dose subjective assessment for the first 11 participants. Therefore, data from only 13 subjects is included for 'alert' and the 'alertness' factors.

5.3.2.4.2 Mental stamina

There was a significant main effect of treatment on ratings of mental stamina [$F(2, 46)=3.98$, $p<0.05$]. Planned comparisons revealed that participants' rated their level of mental stamina as being significantly greater following 75 mg caffeine as compared to placebo [$t(46)=2.75$, $p<0.001$, $d=0.74$], see fig. 5.7a.

5.3.2.4.3 Physical stamina

There was a significant main effect of treatment on ratings of physical stamina [$F(2, 46)=3.60$, $p<0.05$]. Planned comparisons revealed that participants' rated their level of physical stamina as being significantly greater following 75 mg caffeine as compared to placebo [$t(46)=2.60$, $p<0.05$, $d=0.59$], see fig. 5.7b

5.3.2.4.4 Post-individual-task mental fatigue

There was a significant main effect of treatment on ratings of post-individual-task mental fatigue [$F(2, 230)=8.25$, $p<0.001$]. Planned comparisons revealed that participants' rated their level of mental fatigue as being significantly lower following 75 mg caffeine [$t(230)=4.93$, $p<0.001$, $d=-0.83$] and 150 mg caffeine as compared to placebo [$t(230)=4.47$, $p<0.001$, $d=-0.69$], see fig 5.7c.

5.3.2.4.5 Post-individual-task difficulty

There was a significant main effect of treatment on ratings of post-individual-task difficulty [$F(2, 230)=6.985$, $p<0.005$]. Planned comparisons revealed that participants' rated tasks overall as being less difficult following 75 mg caffeine [$t(230)=2.72$, $p<0.01$ $d=-$

0.62] and 150 mg caffeine as compared to placebo [$t(230)=3.10$, $p<0.005$ $d=-0.73$], see fig. 5.7d.

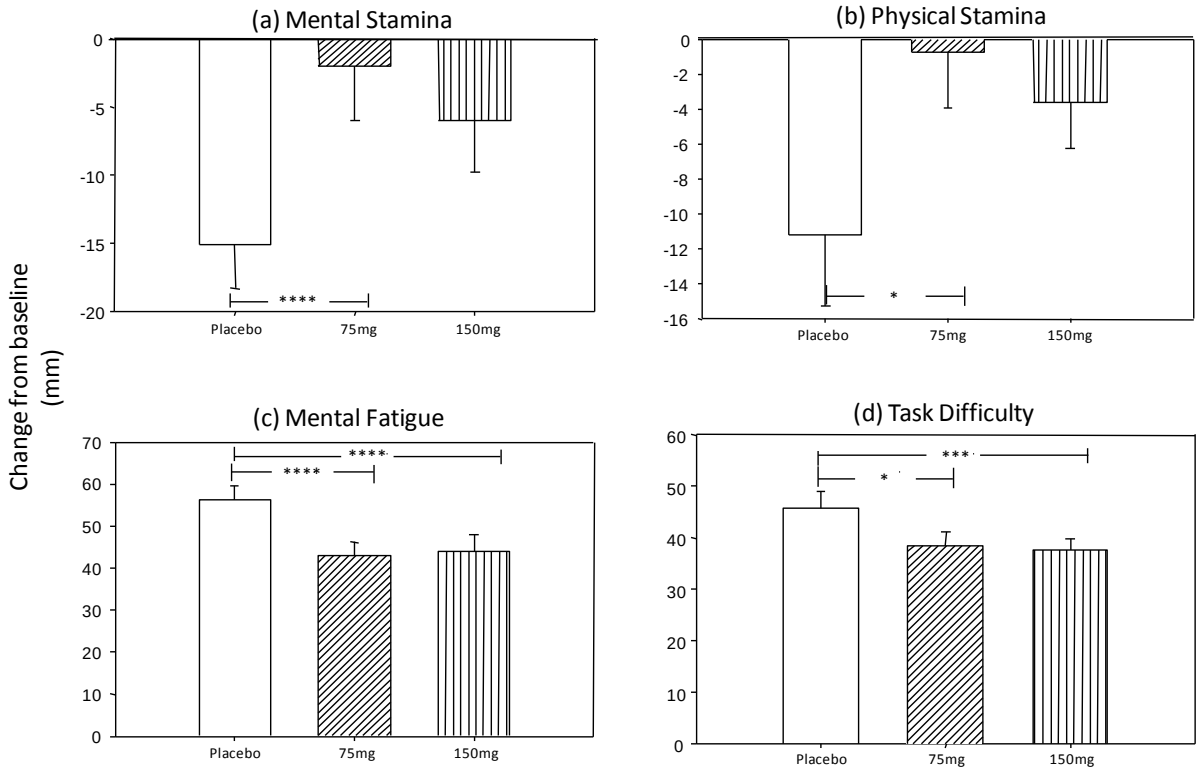


Fig. 5.7. Mean (and SEM) change from baseline scores on mood parameters following placebo, 75 mg caffeine and 150 mg caffeine. Main effects of treatment are shown for (a) mental stamina, (b) physical stamina, (c) mental fatigue and (d) task difficulty significance is compared to the placebo (t-tests calculated with the Mean Squares Error from the ANOVA) (* p<0.05, *** p<0.005, **** p<0.001).

5.3.3 Task related effects

5.3.3.1 Near infrared spectroscopy

Effects of task performance on measures of cerebral blood flow, irrespective of treatment received.

5.3.3.1.1 Oxygenated haemoglobin

A significant main effect of task was observed for oxy-Hb [$F(5, 230)=11.14$, $p<0.001$]. Pairwise comparisons of each task to the control revealed that during the control task oxy-Hb was significantly lower as compared to serial 3s ($p<0.005$), serial 7s ($p<0.005$), serial 17s ($p<0.001$), see fig.

5.3.3.1.2 Deoxygenated haemoglobin

A significant main effect of task was observed for deoxy-Hb [$F(5, 230)=9.704$, $p<0.001$]. Pairwise comparisons (partial Bonferroni corrections) of each task to the control revealed that during the control task deoxy-Hb was significantly lower than during serial 3s ($p<0.01$) and serial 7s ($p<0.05$), see fig. 5.8.

5.3.3.1.3 Total haemoglobin

A significant main effect of task was observed for total-Hb [$F(5, 230)=15.14$, $p<0.001$]. Pairwise comparisons (partial Bonferroni corrections) of each task to the control revealed that during the control task total-Hb was significantly lower than during serial 3s ($p<0.001$), serial 7s ($p<0.005$) and serial 17s ($p<0.001$), see fig. 5.8.

5.3.3.2 Indirect calorimetry

Effects of task performance on metabolism measures, irrespective of treatment received.

5.3.3.2.1 Energy expenditure

A significant main effect of task was observed for energy expenditure [$F(5, 120)=8.470$, $p<0.001$]. Pairwise comparisons (partial Bonferroni corrections) of each task to the control revealed that during the control task, energy expenditure was significantly lower than during serial 3s ($p<0.05$), serial 7s ($p<0.05$) and serial 17s ($p<0.05$), see fig. 5.8.

5.3.3.2.2 Carbohydrate oxidation

A significant main effect of task was observed for carbohydrate oxidation [$F(5, 120)=3.935$, $p<0.005$]. However, pairwise comparisons (partial Bonferroni corrections) of each task to the control revealed no significant differences between tasks for carbohydrate oxidation, see fig. 5.8.

5.3.3.2.3 Fat oxidation

There were no significant task related effects for fat oxidation, see fig. 5.8.

5.3.3.3 Subjective mental fatigue and difficulty

Subjective ratings of mental fatigue and difficulty following individual task performance, irrespective of treatment received.

5.3.3.3.1 Post-individual-task mental fatigue

There was a significant main effect of task on ratings of post-individual-task mental fatigue [$F(5,230)=8.78$, $p<0.001$]. Pairwise comparisons (partial Bonferroni corrections) of each task to the control revealed that participants rated themselves as being significantly less mentally fatigued following the control task as compared to the 3-back ($p<0.005$) and RVIP ($p<0.001$), see fig. 5.8.

5.3.3.3.2 Post-individual-task difficulty

There was a significant main effect of task on ratings of post-individual-task difficulty [$F(5, 230)=40.03$, $p<0.001$]. Pairwise comparisons (partial Bonferroni corrections) of each task to the control revealed that participants rated the control task as being significantly less difficult than serial 3s ($p<0.005$), serial 7s ($p<0.001$), serial 17s ($p<0.001$), 3-back ($p<0.001$) and RVIP ($p<0.001$), see fig. 5.8.

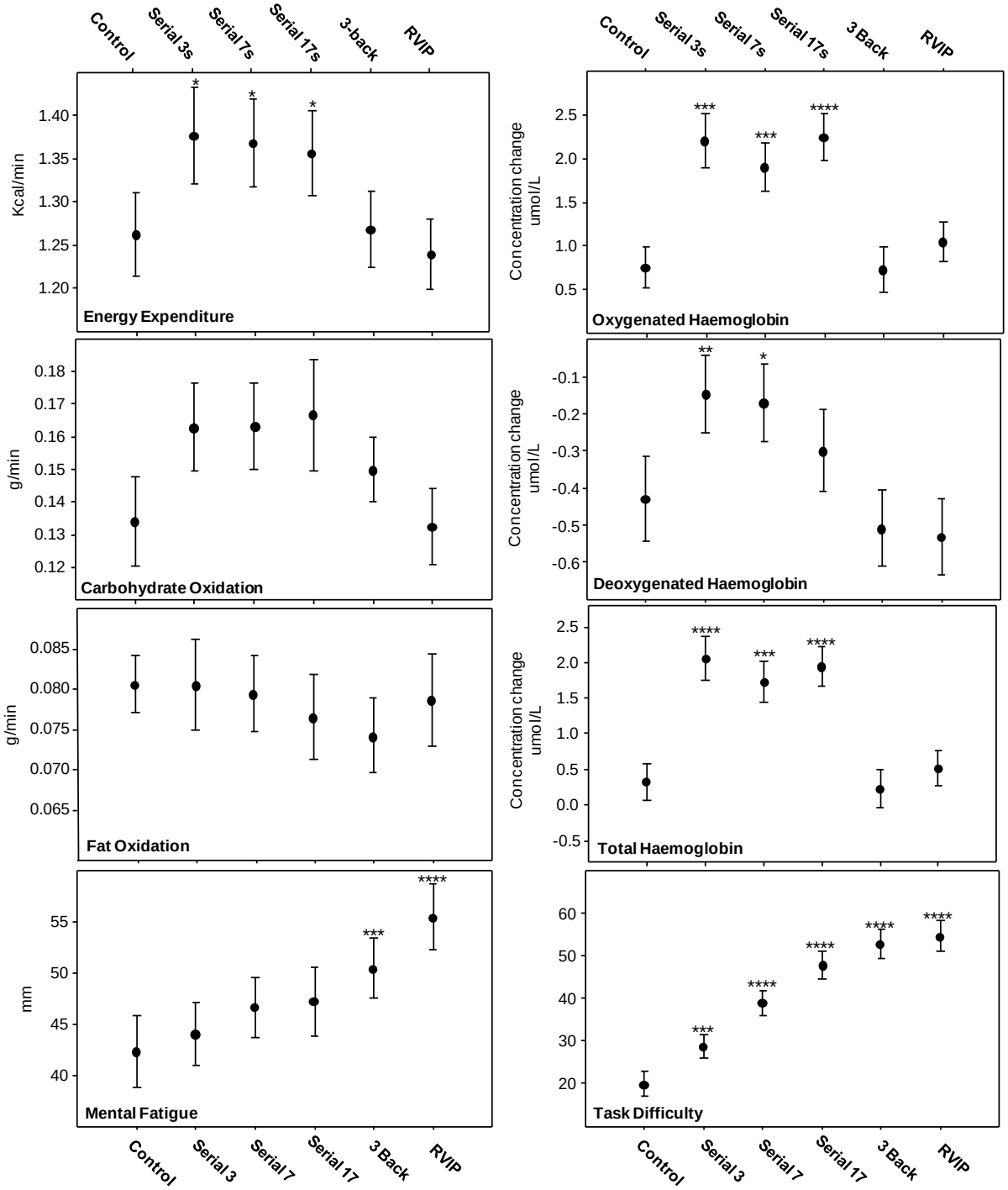


Fig. 5.8. Mean change (and SEM) during each task period, main effects of task are shown for metabolic measures (raw data), cerebral oxygenation (change from baseline) and subjective measure of task difficulty and mental fatigue (raw data). Pairwise comparisons (partial Bonferroni corrections) are shown where each task is compared to the control (*p<0.05, **p<0.01, ***p<0.005, ****p<0.001).

Table 5.1. Baseline and change from baseline scores for serial subtractions, RVIP and 3-back tasks for each treatment. Means ±SEM values are presented with F and p values from the primary ANOVA for main treatment effects. Significant measures are shown in bold.

Measure	*n*	Treatment	Baseline	Post-dose change from baseline score	Treatment effect
Serial 3s subtraction correct (%)	24	Placebo	92.0±1.70	2.46±1.47	F=1.40 p>0.1
		75mg	94.6±1.14	-0.58±1.19	
		150mg	95.4±1.22	0.39±1.19	
Serial 3s subtraction errors (%)	24	Placebo	8.04±1.70	-2.46±1.47	F=1.40 p>0.1
		75mg	5.38±1.14	0.58±1.19	
		150mg	4.57±1.22	-0.39±1.19	
Serial 7s subtraction correct (%)	24	Placebo	94.0±1.19	-3.68±1.81	F<1
		75mg	90.4±1.61	-0.79±1.74	
		150mg	91.4±2.87	0.07±2.96	
Serial 7s subtraction errors (%)	24	Placebo	6.04±1.19	3.68±1.81	F<1
		75mg	9.60±1.61	0.79±1.74	
		150mg	8.60±2.87	-0.07±2.96	
Serial 17s subtraction correct (%)	23	Placebo	89.0±1.95	-0.57±2.04	F<1
		75mg	83.9±2.77	2.62±2.92	
		150mg	85.1±2.46	0.71±2.18	
Serial 17s subtraction errors (%)	23	Placebo	11.0±1.95	0.57±2.04	F<1
		75mg	16.1±2.77	-2.62±2.92	
		150mg	14.9±2.46	-0.71±2.18	
RVIP correct (%)	23	Placebo	65.6±4.52	-6.76±3.87	F<1
		75mg	70.4±3.54	-8.97±3.02	
		150mg	65.8±4.06	-2.72±3.43	
RVIP RT (ms)	23	Placebo	484±10.5	11.1±11.8	F=1.10 p>0.1
		75mg	493±12.1	-8.20±10.4	
		150mg	494±13.4	-3.23±9.13	
RVIP false alarms (%)	23	Placebo	16.0±4.36	-1.94±4.15	F<1
		75mg	10.6±2.20	-1.68±2.34	
		150mg	12.5±2.91	-1.85±1.88	
3-back overall correct (%)	24	Placebo	85.6±2.27	-4.53±3.95	F<1
		75mg	89.1±1.66	-5.59±1.95	
		150mg	88.6±1.28	-8.37±3.13	
3-back overall RT (ms)	24	Placebo	744±54.6	-18.4±32.2	F<1
		75mg	766±52.7	-13.8±27.6	
		150mg	787±52.0	-40.9±35.3	

Table 5.2. Baseline and change from baseline scores for caffeine research visual analogue scales and subjective energy measures for each treatment. Means ±SEM values are presented with F and p values from the primary ANOVA for main treatment effects. Significant measures are shown in bold.

Measure		*n*	Treatment	Baseline	Post-dose change from baseline score	Treat effect
Caffeine research visual analogue scales (mm)	Relaxed	24	Placebo	54.6±2.85	-14.2±4.06	F=1.61 p>0.1
			75mg	53.2±2.55	-6.25±3.43	
			150mg	58.3±2.56	-12.3±3.34	
	Alert	13	Placebo	52.4±3.21	-10.4±6.66	F<1
			75mg	51.9±2.88	-2.54±4.59	
			150mg	56.3±2.87	-4.85±5.06	
	Jittery	24	Placebo	27.2±3.26	17.8±5.80	F=1.19 p>0.1
			75mg	28.8±2.70	9.33±4.38	
			150mg	27.8±3.21	8.75±4.97	
	Tired	24	Placebo	44.7±3.70	5.17±5.65	F=2.91 p=0.065
			75mg	47.0±3.73	-8.08±5.12	
			150mg	39.4±3.86	2.38±4.14	
	Tense	24	Placebo	30.8±3.52	15.0±5.48	F=1.22 p>0.1
			75mg	34.1±2.94	5.04±4.46	
			150mg	31.7±3.35	9.21±3.42	
	Headache	24	Placebo	23.3±3.95	27.7±4.85	F=1.69 p>0.1
			75mg	24.4±4.18	22.9±5.07	
			150mg	21.8±3.92	18.8±4.17	
	Overall mood	24	Placebo	58.6±2.16	-11.9±2.87	F=1.69 p>0.1
			75mg	58.5±2.39	-4.17±3.16	
			150mg	63.2±2.37	-9.83±3.19	
	Mental fatigue	24	Placebo	35.2±3.67	19.9±4.19	F=2.28 p>0.1
			75mg	31.5±2.50	9.79±3.82	
			150mg	33.0±3.04	12.9±3.40	
Energy measures (mm)	Concentration	24	Placebo	56.9±2.42	-14.4±3.41	F=2.42 p=0.1
			75mg	56.0±3.04	-3.29±4.20	
			150mg	57.8±2.94	-6.29±4.38	
	Mental stamina	24	Placebo	57.0±2.42	-15.1±3.19	**F=3.98 p=0.026**
			75mg	54.7±3.02	-2.04±3.95	
			150mg	57.3±2.19	-6.00±3.80	
	Physical stamina	24	Placebo	56.8±3.14	-11.2±4.01	**F=3.60 p=0.035**
			75mg	57.6±2.71	-0.75±3.17	
			150mg	60.3±2.56	-3.62±2.59	
	Mentally tired	24	Placebo	40.3±3.76	13.5±4.50	F<1
			75mg	35.0±2.47	9.58±4.15	
			150mg	36.7±2.85	11.50±3.36	
	Physically tired	24	Placebo	37.5±3.77	9.00±4.83	F=1.66 p>0.1
			75mg	35.7±3.15	0.87±4.88	
			150mg	34.4±3.60	0.71±3.48	

Table 5.3. Raw scores for subjective mental fatigue and difficulty ratings for each task. Means ±SEM values are presented with F and p values from the primary ANOVA of treatment effects and treatment x task interactions. Significant measures are shown in bold.

Measure	*n*	Treat	Post-dose change from baseline score						Treat effect	Treat x task interaction
			Task							
			Control	Serial 3s	Serial 7s	Serial 17s	RVIP	3-back		
Mental Fatigue (mm)	24	Placebo	50.0±4.32	53.2±3.61	56.7±3.60	55.3±4.04	61.3±3.61	61.0±3.51	**F=8.25 p<0.001**	F<1
		75mg	37.2±4.15	39.8±3.86	41.8±4.00	43.2±3.42	52.1±3.89	42.9±3.69		
		150mg	39.8±4.61	39.1±4.18	41.5±4.69	43.2±4.71	53.2±4.43	47.5±4.41		
Task Difficulty (mm)	24	Placebo	22.5±3.74	35.3±4.04	45.7±3.61	52.4±4.47	60.5±4.24	57.8±4.58	**F=6.99 p=0.002**	F<1
		75mg	21.1±3.81	26.1±3.41	35.0±3.78	45.46±3.32	52.3±4.11	50.8±3.43		
		150mg	16.2±2.81	25.0±2.79	36.42±3.34	45.8±4.13	51.1±3.78	50.1±3.50		

5.3.4 Blood pressure and heart rate

5.3.5.1 Systolic blood pressure

There were no significant treatment related differences in systolic blood pressure.

5.3.5.2 Diastolic blood pressure

There were no significant treatment related differences in diastolic blood pressure.

5.3.5.2 Heart rate

There were no significant treatment related differences in heart rate.

5.4 Discussion

The findings here demonstrate that 75 mg caffeine leads to a significant increase in total-Hb during minutes 6-10, 16-20 and 26-30 of the absorption period prior to task performance. In relation to exhaled gas analysis, as compared to placebo, both 75 mg and 150 mg caffeine led to a significant increase in energy expenditure and carbohydrate oxidation during task performance, which increased incrementally with dose. At rest, 75 mg and 150 mg caffeine led to a significant increase in carbohydrate oxidation and a significant decrease in fat oxidation as compared to placebo. The only significant treatment related behavioural effects were on mood measures. The 75 mg dose led to significant increases in ratings of mental and physical stamina and both the 75 mg and 150 mg doses resulted in significant reductions in post-individual-task ratings of mental fatigue and task difficulty.

In terms of haemodynamics, the findings of the present study largely reflected the pattern of change in cerebral oxygenation expected in the face of a cognitive challenge. Here, the observed increase in oxy-Hb coupled with a corresponding decrease in deoxy-Hb relative to the baseline and/or absorption periods (both of which are essentially rest phases here) is consistent with previous NIRS research that has looked at the effects of task performance (Fallgatter & Strik, 1998; Schroeter et al., 2002). However, what is less consistent is that a 75 mg dose of caffeine should lead to a significant increase in total-Hb, during the absorption period, when previous caffeine research consistently reports a

reduction in CBF following caffeine ingestion (Chen & Parrish, 2009a; Laurienti et al., 2003; Mathew & Wilson, 1991; Rack-Gomer et al., 2009). Indeed, in chapter 4, a significant reduction in oxy-Hb was observed as soon as 3 minutes post-treatment with the same dose as used here. In the present study, there was also an absence of an effect of caffeinated treatment following both doses during task performance. This is also surprising as chapter 4 demonstrated that a 75 mg dose engendered a significant reduction in oxy-Hb and a corresponding increase in deoxy-Hb almost throughout the entire task period. One possible explanation for the absence of an effect is the decision to use only habitual caffeine consumers in the present study. In chapter 4 both habitual and non-habitual consumers were recruited and further analysis of the findings on deoxy-Hb in that study revealed that the increase seen was largely as a result of the non-habitual consumer group. Similarly, decreased total-Hb previously shown following 75 mg caffeine was also due to exaggerated effects in the non-consumers, whilst there were no effects in the habitual consumers (Kennedy & Haskell, 2011).

Turning to the treatment related effects on exhaled gas analysis, this is the first study to demonstrate that caffeine administration leads to a significant increase in energy expenditure and carbohydrate oxidation during the completion of cognitive tasks, and that these effects increase incrementally with dose. Caffeine's ability to increase metabolic rate (Acheson et al., 1980; Dulloo et al., 1989) and energy expenditure (Arciero, Gardner, Callesescandon, Benowitz, & Poehlman, 1995; Astrup et al., 1990; Hollands et al., 1981) during periods of rest has been demonstrated previously (although interestingly not in the present study), so too its dose-dependent manner (Astrup et al., 1990). That this effect should also occur during performance of cognitive tasks is therefore unsurprising although hitherto unexplored. With regards the observed significant increase in carbohydrate oxidation during task performance and at rest; previous research has demonstrated that caffeine in combination with glucose leads to an increase in carbohydrate oxidation during exercise (as compared to the administration of glucose alone) (Yeo, Jentjens, Wallis, & Jeukendrup, 2005). However, where caffeine has been administered alone during exercise (Costill et al., 1978), and at rest (Acheson et al., 2004; Acheson et al., 1980), an

increase in fat oxidation has been reported. In the present study, although no effect on fat oxidation was observed during task performance, a significant decrease was seen at rest following caffeine. These inconsistencies may reflect differences in the energy requirements of the brain and muscle as well as dose response effects at rest, given that previous studies administered much higher doses of caffeine than those used here. It is also important to note that the effects at rest in the present study may reflect carryover effects from the baseline cognitive assessment, with caffeine aiding in the preferential oxidation of carbohydrate over fat in order to replenish stores.

The relative absence of treatment effects on cognition is not entirely unexpected as the tasks were selected on the basis of their potential to induce differing levels of metabolic demand, rather than their sensitivity to caffeine. The RVIP task has previously shown sensitivity to caffeine but the demanding paradigm employed may have served to increase arousal to such a level that effects of caffeine were negated, as has previously been observed with a demanding paradigm (Kennedy & Haskell, 2011). In terms of mood, beneficial effects were observed on mental fatigue and ratings of physical and mental stamina as a result of treatment. Improvements to feelings of fatigue and vigour as well as mental energy and energetic arousal have been reported previously as a result of caffeine administration (Lieberman, 2001; Quinlan et al., 2000; Smit & Rogers, 2000). Furthermore, with studies demonstrating that caffeine leads to increased time to exhaustion (Greer et al., 2000; Pasman et al., 1995; Simmonds et al., 2010; Spriet et al., 1992), the increase in feelings of physical stamina observed here, are not counter to expectations. It is unfortunate that in the present study, subjective measures of alertness were only obtained for 13 participants. The small sample size may provide some explanation for the absence of an effect on this measure, since positive effects on measures of alertness have been demonstrated previously, albeit at different doses to those administered here (Quinlan et al., 2000; Rogers et al., 2008).

In addition to treatment related effects, there were also a number of task-related effects. Irrespective of treatment, serial 3s, serial 7s and serial 17s led to a significant

increase in oxy-Hb and total-Hb as compared to the control. Correspondingly, there was a significantly smaller decrease in deoxy-Hb during performance of the serial 3s and serial 7s tasks as compared to the control. In relation to exhaled gas analysis, there was a significant increase in energy expenditure during the serial 3s, serial 7s and serial 17s subtraction tasks as compared to the control. However, participants rated the 3-back and RVIP tasks subjectively as being significantly more mentally fatiguing than the control. In relation to task difficulty, the serial 3s, serial 7s, serial 17s, 3-back and RVIP tasks were all rated as being significantly more difficult than the control.

These task related effects (irrespective of treatment) are of particular interest in that NIRS and ICa findings presented a similar pattern of effects. Overall and irrespective of treatment, the serial subtractions tasks led to the most pronounced increases in oxy-Hb, the smallest reductions in deoxy-Hb and augmented energy expenditure and carbohydrate oxidation levels to the highest degree (although the findings in terms of carbohydrate oxidation failed to reach significance). By comparison, both the 3-back and RVIP tasks led to changes in the above parameters that were more in-keeping with those of the control task. The study by Kennedy et al. (2016) (supplemental data) found a strikingly similar pattern of effects using a similar methodology as here. They observed that the 3-back task was found to elicit metabolic changes (to total energy expenditure and carbohydrate oxidation), and cerebral blood flow changes (oxy-Hb), that were in-keeping with those of the control task as compared to that of the serial 3s, serial 7s and serial 17s. Importantly, this finding was despite the 3-back task being subjectively rated as more difficult and mentally fatiguing than the subtractions tasks (just as they were in the present study, with the addition of the RVIP). In the case of energy expenditure, this demonstrated that not only the somatic demands of the task had bearing on the increase observed, but also the cognitive demands, since the control task was matched to the most somatically demanding serial 3s subtraction task. The changes with regards carbohydrate and fat oxidation also followed a very similar pattern to that of Kennedy et al. (2016). As with energy expenditure, the 3-back (and also in the present study the RVIP task) was more closely matched to that of the control and there was an increase in carbohydrate

oxidation and a decrease in fat oxidation as the difficulty of the serial subtraction calculations increased, despite the somatic demands of the subtraction tasks decreasing (fewer key presses per minute). In the present study, however, the findings failed to reach significance. With regards to the CBF effects, again for oxy-Hb a similar pattern to that of Kennedy et al. (2016) was observed with the serial subtractions tasks eliciting a significantly greater increase as compared to the control and the 3-back, despite being rated as less cognitively demanding than the 3-back task.

When task related findings are considered in conjunction with the subjective assessments of task difficulty and ratings of mental fatigue engendered by each task, these findings are perhaps, contrary to expectation. Although all of the tasks were rated as being significantly more difficult than the control task, it was the 3-back and RVIP tasks that elicited the highest subjective difficulty rating. In addition, the 3-back and RVIP tasks were the only two tasks rated as being significantly more mentally fatiguing than the control. That the subjective and physiological assessments of a task should provide such contrasting results could, in part, be due to the nature of the tasks themselves. A previous study undertaken in the same research facility (Jackson et al., 2012, internal communication) that looked at the subjective ratings of Serial 3s, Serial 7s and the RVIP task demonstrated that whilst the RVIP was rated as requiring significantly more hard work and mental effort than the serial 3s task, it required significantly less focus. Unfortunately continuous heart rate measurements were not taken in the present study, as this may have provided a further physiological measure of the level of demand each task was placing on the subject (Mehler, Reimer, & Coughlin, 2012). Despite this, the findings of the present study clearly demonstrate that the serial subtractions tasks are the most physiologically demanding (in terms of energy expenditure) and neurally demanding as they elicit the greatest increase in oxygenated haemoglobin and total haemoglobin as well as the greatest increase in deoxygenated haemoglobin, which taken together could reflect both an increase in cerebral blood flow and oxygen extraction. These findings suggest that measuring CBF in conjunction with the metabolic effects provide a more comprehensive overview of the resources required during cognitive task performance and

indeed complement each other in their analysis of the demands of individual tasks. They have also demonstrated that subjective measures are not always accurate in determining specific features of task difficulty and should ideally, be used alongside a physiological measure of demand.

There were some limitations to the present study, however. Data from only 13 participants was included in the metabolism analysis. This was because participants were excluded as their respiratory exchange ratio (RER) values fell outside of the expected range for a fasted, resting state. The expected value for this state is usually between 0.7-0.9 (calculated as the ratio between the amount of carbon dioxide produced and the amount of oxygen consumed). Indeed, for many of the excluded participants, values were >1, which would normally be observed during intense exercise. The exact reason for these anomalous values is not wholly apparent; however, it may relate to the nature of the study and the equipment used. During indirect calorimetry measurements, participants are required to wear a mask over their nose and mouth and to breathe through their mouth (expelled gas is collected into a turbine which is housed within the mask and positioned directly in front of the mouth). There are two issues with this; the mask may not securely fit the face, thereby leaving gaps for expired air to escape (despite every attempt to avoid this through the use of differing mask sizes and plugging gaps in the mask manually). The second is that breathing through their mouth is a counterintuitive action, since at rest breathing through the nose would be a more natural response. It is possible that in the present study participants forgot to breathe through their mouth or simply chose not to (the monitoring of this was made difficult as participants were facing away from the researcher). This could lead to build-up and false elevation of VCO_2 readings. The significance of both of these factors therefore is the influence they bear on the RER readings, since this, as previously explained, is calculated using the values of expired CO_2 and inspired O_2. Another possibility is that the participants may not have fasted as this would also lead to an elevation of the RER value. Although participants were asked upon the morning of their visit if they had remembered to abstain from eating or drinking anything other than water and all confirmed that they had, there is always the

possibility that this procedure was not adhered to. Future studies looking at the metabolic effects of task performance may benefit from the use of a mouthpiece and nose clip that directs participants to breathe through their mouth and removes the need for a facemask. The issue of fasting could be addressed by requesting that participants give a blood sample upon arrival, allowing glucose levels to be determined in order to confirm that participants are fasted.

The absence of heart rate readings during performance of each task meant that there was no objective measure of demand. Previous research has observed that heart rate is able to differentiate between levels of demand, with more cognitively demanding tasks leading to an increase in heart rate (Mehler et al., 2012). Using this measure during tasks in the present study could have gone further to provide a clearer picture in terms of which tasks were found the most demanding.

This is the first study to use NIRS and indirect calorimetry in conjunction with an assessment of cognitive performance in the presence of caffeine. It has demonstrated that a 75 and a 150 mg dose of caffeine leads to an incremental increase in energy expenditure and carbohydrate oxidation during cognitive task performance. It has also demonstrated that irrespective of treatment, the metabolism of central and peripheral substrates follow a similar pattern of effects during specific task performance. Future studies would benefit from including a continuous measure of heart rate as well as subjective measures of task 'experience' that provide a clearer distinction of the of level of effort/demand that a task requires.

Chapter 6: Effects of dietary nitrate supplementation (beetroot juice) during incremental exercise, on cerebral haemodynamics, cognitive performance and subjective fatigue.

6.1 Introduction

Nitric oxide (NO), an autocrine and paracrine signalling molecule, is an important modulator of a number of physiological processes, such as platelet function (Radomski, Palmer, & Moncada, 1990), host defence, neurotransmission, peripheral vasodilation (Moncada & Higgs, 1993) and cerebral vasodilation (Toda et al., 2009). Endogenously, NO is generated through the oxidation of L-arginine by a family of enzymes known as NO synthases (NOSs) (Knowles & Moncada, 1994; Knowles, Palacios, Palmer, & Moncada, 1989; Palmer, Ashton, & Moncada, 1988). NO can also be generated via an alternative pathway, independent of NOS, either in vivo under hypoxic conditions (Lundberg & Weitzberg, 2010) or following the intake of supplementary nitrate (NO_3^-) in its pharmacological form (Lundberg & Govoni, 2004). NO can also be stimulated through the intake of foods (predominantly fruit and vegetables) containing high levels of nitrate (Webb et al., 2008). Via this route, nitrate is reduced to nitrite (NO_2^-) in the mouth by oral bacteria on the posterior of the tongue. Nitrite is then reduced to NO in the stomach and the remainder alongside the remaining nitrate is absorbed by the small intestine into circulation. The nitrate that is not excreted in urine (approx. 25 %) is then taken up by salivary glands and concentrated into saliva, reduced to nitrite and absorbed once again from the intestine, where it is reduced to NO (Lundberg, Weitzberg, & Gladwin, 2008).

Beetroot is a member of the *chenopodiaceae* family, a class of vegetables that has been shown to accumulate comparatively large levels of nitrate, with beetroot itself containing 1727 mg/kg (Santamaria et al., 1999). Following consumption of nitrate-rich food (500 ml beetroot juice), a 16 fold increase in nitrate has been demonstrated, peaking at 90 minutes post-consumption, with a corresponding 2-fold increase in plasma nitrite levels, peaking at 3 hours post-consumption (Webb et al., 2008). Supplementation of dietary nitrate in humans (in the form of beetroot juice) has led to effects consistent with

those observed as a result of increases in endogenous NO synthesis, such as improvements in the vasculature. Joris and Mensink (2013) observed that beetroot juice led to a reduction of post-prandial impairment in flow-mediated dilation in healthy overweight and slightly obese men. In healthy populations, reductions in blood pressure have been observed (Kapil et al., 2010; Vanhatalo et al., 2010; Webb et al., 2008; Wylie et al., 2013) with nitrate supplementation also leading to protection against endothelial dysfunction in the forearm, and inhibition of platelet aggregation (Webb et al., 2008). Evidence from animal studies have also supported the nitrite-NO pathway as a means of positively modulating CBF (Rifkind et al., 2007) and neurovascular coupling after a challenge (Piknova, Kocharyan, Schechter, & Silva, 2011). It is believed this method of NO production supplements the oxygen dependent L-arginine/NO pathway and may prevail under conditions of hypoxia (Lundberg & Weitzberg, 2010), such as is observed in skeletal muscle during exercise.

Recently there have been a number of studies assessing the impact of dietary nitrate supplementation during the implementation of exercise protocols. Bailey et al. (2009) administered 500 ml of beetroot juice (BR) per day (nitrate 5.5 mmol per 500 ml) over a period of 6 consecutive days to young recreationally active males. They demonstrated that beetroot juice leads to a significant reduction in the amplitude of pulmonary VO_2 during moderate intensity exercise, alongside a significant (13 %) reduction in the amplitude of muscle deoxy-Hb (as measured by NIRS); inferred as being indicative of reduced oxygen extraction. BR also increased time to exhaustion during severe exercise. The attenuation of deoxy-Hb, in conjunction with the change in VO_2, was taken as evidence of reduced aerobic energy turnover or muscle energy utilisation. Increases in the time to exhaustion following BR during high intensity exercise have since been demonstrated (Bailey et al., 2010; Breese et al., 2013; Wylie et al., 2013), so too reductions in the oxygen cost of sub-maximal exercise following both chronic (Bailey et al., 2010; Lansley, Winyard, Fulford, et al., 2011; Vanhatalo et al., 2010) and acute (Vanhatalo et al., 2010; Wylie et al., 2013) BR supplementation. It has also been shown to improve cycling performance (Lansley, Winyard, Bailey, et al., 2011), rowing

performance (Bond, Morton, & Braakhuis, 2012) and aspects of exercise recovery following a bout of eccentric exercise (Clifford, Bell, West, Howatson, & Stevenson, 2016).

However, the effects of BR are not confined to the periphery as modulation of the cerebral vasculature has also been observed. Presley et al. (2011) looked at the effects of a high nitrate diet (including 500 ml BR) on cerebral perfusion (via arterial spin labelling magnetic resonance imaging) in older adults. In this crossover study, participants received a diet high in fruits and vegetables plus 500 ml of BR at breakfast (providing a high nitrate diet of 12.4 mmol nitrate) or a diet low in fruits and vegetables with no supplementary BR (providing a low nitrate diet of 0.089 mmol nitrate). In addition to significantly elevated nitrate and nitrite levels, the high nitrate diet led to a significant increase in cerebral perfusion in the white matter of the frontal lobe as compared to that following the low nitrate diet. The effects of BR supplementation have also been assessed on cerebral perfusion during cycling at 40 %, 60 % and 80 % VO_{2peak}, in healthy young adults. Following 500 ml acute beetroot supplementation, a significant reduction in cerebrovascular resistance index (CRI) (calculated as middle cerebral artery mean blood velocity (MCA Vmean)/mean arterial blood pressure, as measured by Transcranial Doppler) was observed, at rest and during all workout intensities (Bond et al., 2013). Rattray et al. (2015) assessed the effects of an acute low dose (140 ml) of BR supplementation on MCA Vmean (via Transcranial Doppler), during cycling performance whilst simultaneously measuring cognitive performance on the Stroop task. They demonstrated that MCA Vmean was significantly elevated during higher intensity exercise performance (75 % and 85 % of heart rate reserve), but particularly so during performance of the cognitive task. Although no significant behavioural effects were observed, there was an improvement in performance accuracy on the Stroop task that just failed to reach significance ($p=0.059$). The CBF velocity (via Transcranial Doppler) effects of BR administration have also been assessed during cognitive task (Stroop) performance in hypoxia in healthy young males. However, here, Lefferts et al. (2015) observed no effects on neurovascular coupling or cognitive performance following acute beetroot (70 ml) supplementation.

A study by Wightman et al. (2015) assessed the cognition and cerebral oxygenation enhancing effects of dietary nitrate supplementation in healthy young adults. It was observed that 450 ml BR led to an initial increase in CBF (total-Hb) at the onset of the task period during an assessment of mental arithmetic (serial 3s subtractions), with a subsequent reduction in CBF during RVIP, a task that has been associated with smaller changes in CBF in previous studies (Kennedy, Wightman, et al., 2010) as well as in chapter 5 of this thesis. As the task period progressed, however, CBF was also reduced for a later completion of serial 3s. In terms of cognitive performance, the only finding was that BR led to a significant improvement in performance of serial 3s subtractions. Other studies that have assessed the effect of BR on cognitive performance, have demonstrated mixed results. Supplementation has led to improvements in reaction time on the Stroop task during exercise (Thompson et al., 2015), but also no effect on performance (on tasks including the Stroop and RVIP tasks), in the presence (Rattray et al., 2015) and absence of exercise in healthy older adults (Kelly et al., 2013). Beetroot supplementation has also led to an absence of effects on the Stroop task under hypoxic conditions (Lefferts et al., 2015).

The purpose of the present study was therefore to extend the methodological approach used in previous chapters by measuring the haemodynamic effects of cognitive task performance, but introducing an element of exercise to the protocol, with the aim of increasing levels of demand both peripherally and centrally. It was also anticipated that this methodology would further current knowledge of the peripheral and central effects of BR by combining exercise and cognitive function protocols, in order to determine if one impacts upon the other in the presence of BR. This would be achieved through measurement of NIRS-related cerebral haemodynamics, cognitive function and subjective mental fatigue and energy levels, taken before during and after simultaneous exercise at a range of intensities (50 %, 70 % and 90 % VO_{2peak}). It was hypothesised that, in comparison to placebo, BR supplementation would elevate plasma nitrite levels, positively modulate cerebral oxygenation during exercise and at rest and improve subjective mental fatigue and energy levels.

6.2 Method

6.2.1 Participants

Sixteen healthy young, recreationally active male participants between the ages of 18 and 35 (mean age 23, SD 4.1; BMI 24.2, SD 2.9) were recruited. The study was approved by Northumbria University's School of Psychology and Sport Sciences' ethics committee and conducted in accordance with the Declaration of Helsinki. Prior to participation, volunteers were required to sign an informed consent form. A general health screen (appendix B for example) informed volunteers that they would not be eligible to take part if they had a history of neurological, vascular or psychiatric illness or a history or current diagnosis of drug or alcohol abuse. They would also be ineligible if they had a current diagnosis of depression or anxiety, anaemia, high blood pressure, a heart or respiratory disorder, type 1 diabetes, phenylketonuria, a history of head trauma, migraines, learning difficulties, dyslexia or ADHD. As blood samples would be taken as part of the study, volunteers were informed they would not be eligible to take part if any of the following applied; they had an active infection, were HIV antibody positive, they had a past or present diagnosis of hepatitis, had jaundice within the last year, haemophilia or any similar blood clotting disorder. All participants reported that they were in good health, had normal or corrected-to-normal vision and had no known food intolerances or sensitivities. Additionally, they were not currently taking any dietary supplements or medication, were not colour-blind and did not smoke.

6.2.2 Design and treatment

A randomised, double-blind, counter-balanced, within subjects, placebo-controlled design was utilised. Participants attended two study visits and at each received one of the following: 450 ml organic beetroot juice, containing 5 mmol nitrate (Beet It, James White Drinks, Ipswich, UK) and 50 ml low calorie blackcurrant cordial or placebo (50 ml low calorie blackcurrant cordial, 45 ml pressed apple juice and 405 ml water). Pressed apple juice was added to the placebo as Beet It contains 10 % apple juice (and there was no interest in the effects of apple juice on performance of any of the measures in the current study). Each treatment was administered in the form of a drink. The order in which

participants received each treatment was determined by Latin square and random allocation to treatment order.

6.2.3 Blood plasma nitrite levels

Venous blood samples were collected into lithium heparin tubes for determination of plasma nitrite. Samples were centrifuged at 4000 rpm for 10 minutes at 4 °C within 3 minutes of collection. Plasma was collected and stored at -80 °C until further analysis for nitrite by chemiluminescence using the procedures described by Bailey et al. (2009). Samples were taken upon arrival, following the 90-minute post-treatment absorption period and immediately after cycling to 90 % $VO_{2peak.}$

6.2.4 Physiological, cognitive and mood measures

The present chapter formed part of a larger study and consequently data was collected that will not be reported here. These included measures of electromyography (EMG), muscle oxygenation (via NIRS), heart rate, pulmonary ventilation, VO_2, VCO_2 and RER (via indirect calorimetry) all of which were taken during exercise performance. Blood lactate and ratings of perceived exertion, which were taken before, during and after exercise. Measurements of blood pressure and assessments of mood via the Brunel Mood Scale, which were taken at various stages before and after exercise.

6.2.4.1 Near infrared spectroscopy measurements

Please see chapter 2 for a full description of the NIRS method used, which is identical to that used in the present chapter.

6.2.4.2 Cognitive and subjective fatigue assessment

All cognitive measures were delivered using COMPASS. Please see chapters 2 and 3 for a description of COMPASS and an explanation of tasks and mood assessments listed here but not described in full below. The tasks were chosen based on their ability to activate the pre-frontal cortex (Lawrence et al., 2002; Schroeter et al., 2002). A response pad was used for participant responses to all tasks and during exercise was securely

mounted onto the handle bars of the cycle ergometer. The tasks were completed once at baseline and four times post-dose; during a pre-exercise period, whilst cycling at 50 % VO_{2peak}, whilst cycling at 70 % VO_{2peak} and after exercise. The tasks were identical at each period and followed the same order; RVIP, Stroop. They differed only in their duration (baseline tasks were 2 minutes and all post dose tasks were 9 minutes). After the completion of each set of tasks at each period, participants were required to complete subjective mental fatigue and energy level ratings.

6.2.4.2.1 RVIP

Please see chapter 2 for a description of this task. This task was scored for percentage of target strings correctly detected, average reaction time for correct detections and percentage of false alarms.

6.2.4.2.2 Stroop

Please see chapter 2 for a description of this task. This task was scored for percentage accuracy and reaction time.

6.2.4.2.3 Subjective fatigue

Following completion of each set of cognitive tasks, participants were presented with the questions “how mentally fatigued do you feel right now” and “how energetic do you feel right now” and asked to rate their current state by marking a vertical line on a 100 mm line on an A4 piece of paper with the end points labelled ‘not at all’ (left hand end) and ‘extremely’ (right hand end).

6.2.5 Procedure

Participants were required to attend the laboratory on three separate occasions. The first visit was a screening session where participants were informed about the nature of the study, its requirements and its restrictions. Informed consent was obtained and eligibility to participate was confirmed and familiarisation with the tasks to be administered on the study days was conducted. At this visit participants also completed an incremental exercise test to determine VO_{2peak}. Following a 5-minute rest period, participants

completed 3 minutes of 'unloaded' (20 W) cycling, after which the work rate was increased by 30 W/min until volitional exhaustion. VO_{2peak} was determined as the highest 30 second mean VO_2 value achieved prior to exercise termination. The remaining two study visits were identical to each other, with the exception of the treatment administered. On each day, participants attended the lab at 8 am following an overnight 12-hour fast. Participants were required to abstain from alcohol for 24 hours and they were also expected to avoid strenuous exercise for 24 hours prior to each assessment. In addition, participants were provided with a list of nitrate rich foods which they were asked to avoid for 36 hours prior to each visit. Upon arrival, baseline blood plasma nitrite samples were taken. Following this, the NIRS headband was fitted and NIRS recording began. Participants initially sat quietly for 5 minutes whilst NIRS readings stabilised, before making a baseline completion of the cognitive tasks, and subjective mental fatigue/energy level measures. A 10-minute baseline assessment of NIRS readings were then taken whilst participants sat quietly and watched a non-stimulating home improvement DVD. Participants were then required to take their treatment for the day. Due to the treatment being administered as a 500 ml drink, 10 minutes was allowed for treatment consumption. Following a (further) 80-minute absorption period (during which time NIRS recording continued whilst participants watched the DVD), participants completed a second set of the cognitive tasks and subjective measures. Blood plasma nitrite samples were taken for a second time before the exercise test began. During the test, participants were required to cycle on a cycle ergometer (Velotron, Dynafit Pro, RacerMate Inc, Seattle, USA), initially for 3 minutes 'unloaded' (20 W), followed by two, 20 minute incremental stages at work rates required to elicit ~50 % and ~70 % VO_{2peak} and a final work rate corresponding to 90 % VO_{2peak} which was continued to task failure (defined as a fall in pedal rate of >10 rpm below self-selected cadence). Whilst cycling during the 50 % and 70 % VO_{2peak} stages, participants performed a set of the cognitive tasks and immediately upon completion of each stage, the subjective measures. Following the 90 % VO_{2peak}, no cognitive tasks were performed; however, subjective measures were still obtained upon completion of this stage. A final blood plasma nitrite sample was then taken, followed by

a final completion of the cognitive tasks (in the absence of exercise) and subjective measures before participants were able to leave (see fig 6.1 for more details of procedure and task duration). Participants returned for their next study visit following (at least) a 1-week washout period.

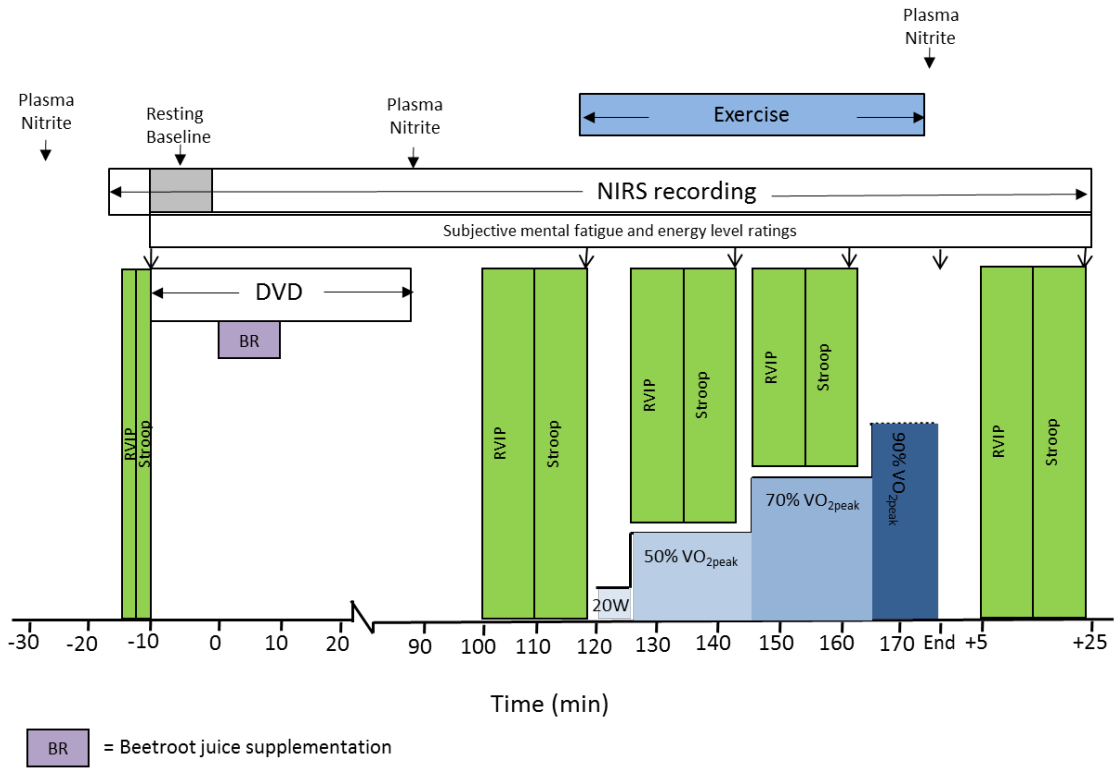


Fig. 6.1. Timeline representing flow of study day.

6.2.6 Statistics

Blood plasma nitrite was analysed by 2-way repeated measures ANOVA (treatment; (beetroot juice or placebo) X epoch (pre-supplementation, post-supplementation/pre-exercise, post-exercise)). Significant differences were further explored by Bonferroni adjusted pairwise comparisons.

Prior to the primary NIRS analysis a within subjects ANOVA was carried out with left/right optode included as a factor to examine any treatment related hemispheric differences in response. As there were no interpretable interactions involving this factor the data from the two channels were averaged for the analysis.

For the primary NIRS analysis, the question under investigation was how beetroot juice would modulate cerebral oxygenation parameters over time as compared to placebo. Data for oxy-Hb, deoxy-Hb and total-Hb was averaged across 10 minute epochs during the absorption period and 9 minute epochs during the task period and baseline adjusted to the last 5 minutes of the post-task resting pre-treatment period. Data was then analysed by 2-way repeated measures ANOVA (treatment (beetroot juice or placebo) X epoch (9 x 10 minute epochs for absorption period and 8 x 9 minute epochs for task period)). Significant treatment related interactions were described with reference to *a priori* planned comparisons, where the active treatment was compared to placebo at each epoch utilising t tests calculated with the Mean Squares Error from the ANOVA (Keppel, 1991). In order to reduce the potential for Type I errors only those planned comparisons associated with a statistically significant difference on the initial ANOVA are reported. In addition, only those instances where a consistent pattern of significant differences are maintained across epochs are identified as reportable significant effects.

In order to identify if effects were specific to task performance, a further analysis was conducted whereby the task period was analysed alone by 2-way repeated measures ANOVA (treatment (as above) X task (RVIP before exercise, Stroop before exercise, RVIP 50% VO_{2peak}, Stroop 50% VO_{2peak}, RVIP 70% VO_{2peak}, Stroop 70% VO_{2peak}, RVIP after exercise, Stroop after exercise). Planned comparisons were conducted as per primary analysis, documented above.

Secondary analysis of the data focused on the task period, included exercise level as a factor and was aimed at assessing the effects of treatment in more detail. This was achieved through the use of shorter-duration epochs in order to identify if treatment related effects were confined to a specific stage within the study and/or a specific time-point within a task. For oxy-Hb, deoxy-Hb and total-Hb, data was averaged across 3 minute epochs within each task and baseline adjusted to the last 5 minutes of the post-task resting pre-treatment period. It was then analysed by repeated measures ANOVA (treatment (as above) X task (RVIP, Stroop) X exercise level (prior to exercise, 50%

VO_{2peak}, 70% $VO_{2peak,}$ after exercise) X epoch (3-minute time points)). Planned comparisons for secondary analysis were conducted as per primary analysis, documented above.

To assess the possibility of any on-day differences in cognitive performance, subjective mental fatigue and energy level measures, paired samples t-tests were conducted on baseline data. Any significant differences were further explored with Bonferroni corrected pairwise comparisons.

Cognitive performance, subjective ratings of mental fatigue and energy levels were analysed as 'change from baseline' by 2-way repeated measures ANOVA (treatment (beetroot juice or placebo) X repetition (before exercise, 50% VO_{2peak}, 70% VO_{2peak}, after exercise)). Significant treatment related effects were described with reference to *a priori* planned comparisons (as above) where the active treatment was compared to placebo.

6.3 Results

6.3.1 Plasma nitrite

Due to missing data points and one instance of anomalous data, only 13 data sets are included in this analysis.

There was a significant treatment x time point interaction, whereby plasma nitrite was significantly elevated pre and post-exercise following beetroot supplementation as compared to placebo (F (1,12)=30.96, p<0.005). Bonferroni corrected pairwise comparisons revealed that post-dose/pre-exercise dietary nitrite values were significantly elevated as compared to placebo (p=0.001), so too were post-dose/post-exercise nitrite values (p=<0.01). There was no significant difference between pre-dose dietary nitrite values for beetroot and placebo treatments, see fig. 6.2.

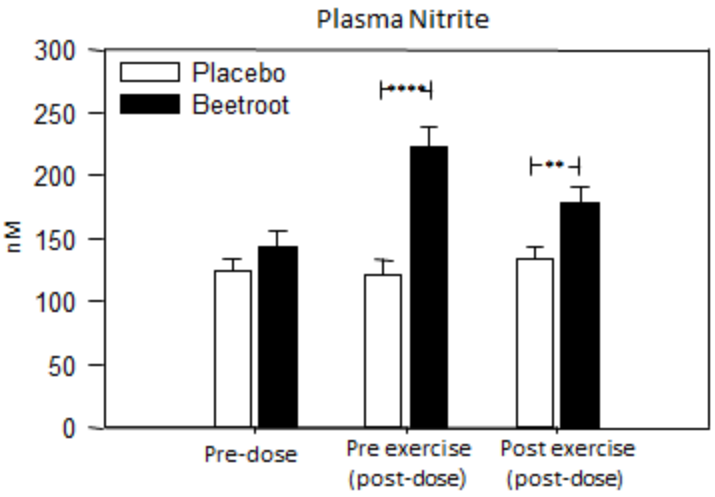


Fig.6.2. Mean (and SEM) nitrite values pre-dose, pre-exercise (post-dose) and post-exercise (post-dose) following either beetroot juice or placebo. Treatment x time point effects are shown, significance is compared to placebo (**** p<0.001, ** p<0.01).

6.3.2 Near infrared spectroscopy

6.3.2.1 Primary analysis

Effects of treatment on cerebral blood flow over time.

6.3.2.1.1 Oxygenated haemoglobin

A significant main effect of treatment was observed for oxy-Hb [F(1, 240)=6.29, p<0.05, d=0.18] whereby beetroot juice led to significantly increased oxy-Hb overall as compared to placebo, see fig. 6.3a. A significant interaction effect (treatment x epoch) was also observed [F(16, 240)=2.09, p<0.05]. Planned comparisons revealed that oxy-Hb was significantly higher following beetroot as compared to placebo during the absorption period at minutes 1-10 [t(240)=3.90, p<0.001, d=1.18], 11-20 [t(240)=4.92, p<0.001, d=1.48], 21-30 [t(240)=4.79, p<0.001, d=1.61], 31-40 [t(240)=3.60, p<0.001, d=1.14], 41-50 [t(240)=3.64, p<0.001, d=1.0], 51-60 [t(240)=2.54, p<0.05, d=0.63], 61-70 [t(240)=2.91, p<0.005, d=0.63]. It was also significantly higher during RVIP 50 % VO_{2peak} [t(240)=2.63, p<0.01, d=0.24] and during RVIP [t(240)=3.69, p<0.001, d=0.36] and Stroop [t(240)=5.33, p<0.001, d=0.56] after exercise completion, see fig. 6.4a.

6.3.2.1.2 Deoxygenated haemoglobin

A significant main effect of treatment was observed for deoxy-Hb [$F(1, 240)=8.24$, $p<0.05$, $d=-0.21$] whereby beetroot juice led to a significant reduction in deoxy-Hb overall as compared to placebo, see fig. 6.3b. For graph representing treatment X epoch effects see fig. 6.4b, there were no significant effects of this interaction.

6.3.2.1.3 Total haemoglobin

A significant main effect of treatment was observed for total-Hb [$F(1, 240)=4.63$, $p<0.05$, $d=0.14$] whereby beetroot juice led to significantly increased total-Hb overall as compared to placebo, see fig. 6.3c. A significant interaction effect (treatment x epoch) was also observed [$F(16, 240)=2.26$, $p<0.005$]. Planned comparisons revealed that total-Hb was significantly higher following beetroot as compared to placebo during the absorption period at minutes 1-10 [$t(240)=3.55$, $p<0.001$, $d=1.18$], 11-20 [$t(240)=4.70$, $p<0.001$, $d=1.51$], 21-30 [$t(240)=4.65$, $p<0.001$, $d=1.53$], 31-40 [$t(240)=3.21$, $p<0.005$, $d=1.0$], 41-50 [$t(240)=3.33$, $p<0.005$, $d=0.92$], 51-60 [$t(240)=1.99$, $p<0.05$, $d=0.51$], 61-70 [$t(240)=2.31$, $p<0.05$, $d=0.48$]. It was also significantly higher during RVIP 50 % VO_{2peak} [$t(240)=2.23$, $p<0.05$, $d=0.16$] and during RVIP [$t(240)=3.44$, $p<0.001$, $d=0.30$] and Stroop [$t(240)=5.27$, $p<0.001$, $d=0.50$] after exercise completion, see fig. 6.5.

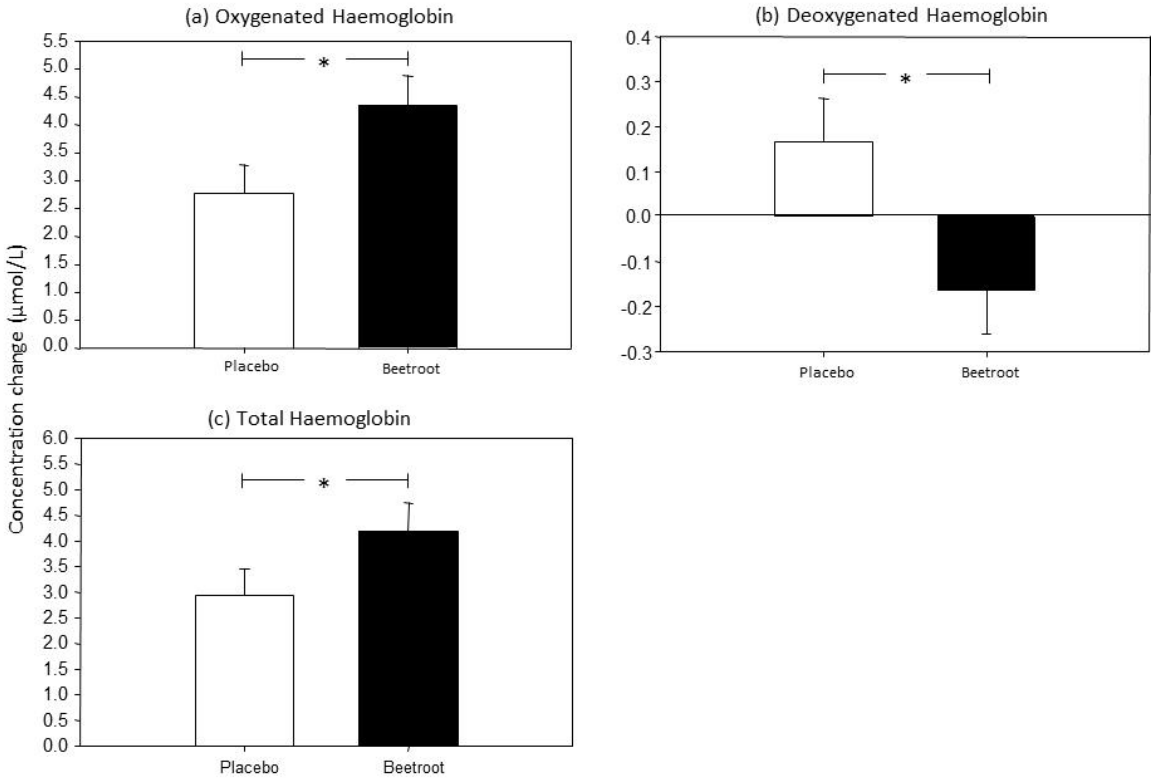


Fig. 6.3 Concentration changes of (a) oxy-Hb, (b) deoxy-Hb and (c) total-Hb overall (absorption and cognitive task period) following placebo and beetroot supplement. Means and SEM are presented as change from pre-treatment, resting baseline. Main effects of treatment are shown (t-tests calculated with the Mean Squares Error from the ANOVA) (* $p<0.05$).

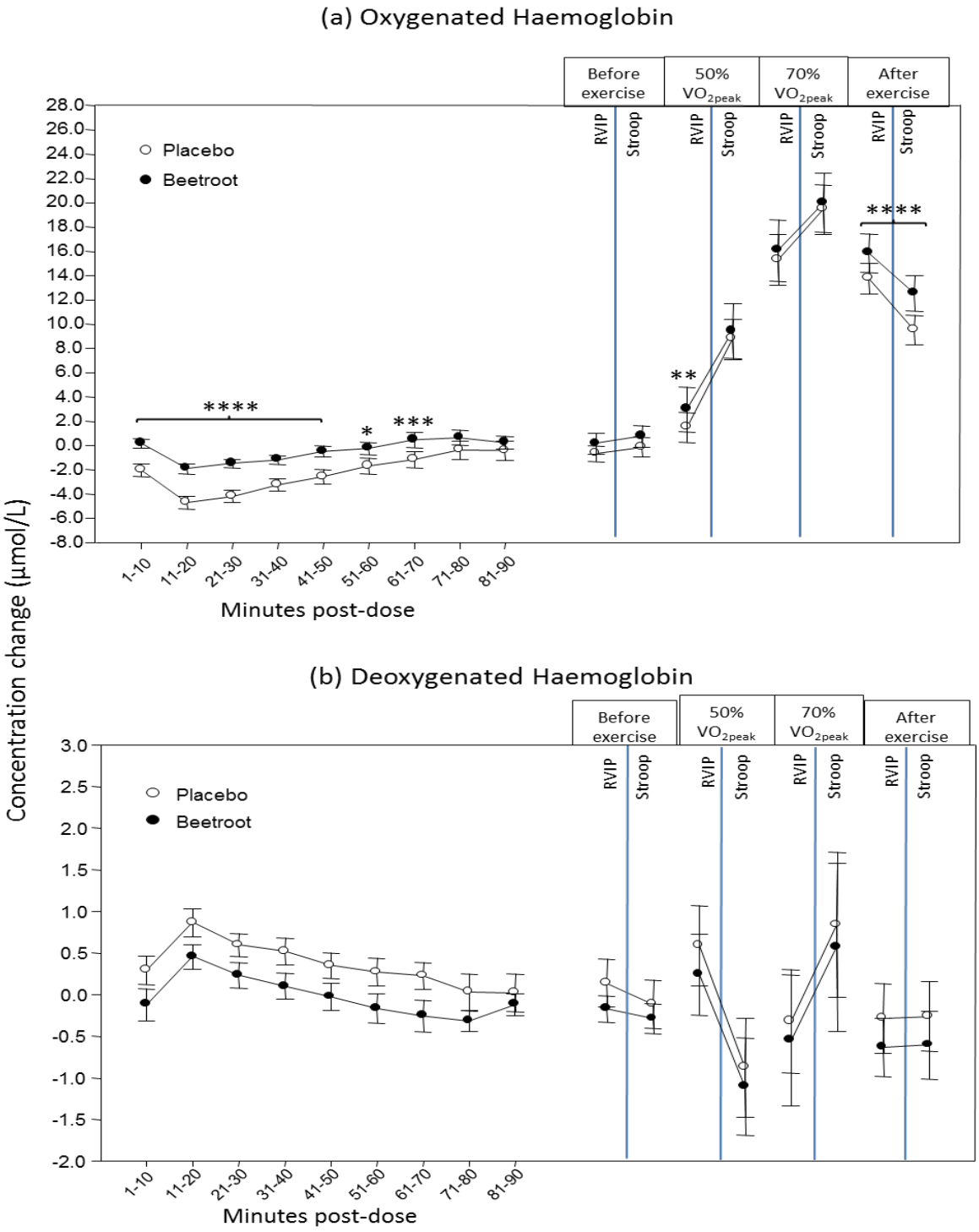


Fig. 6.4. Concentration changes of (a) oxy-Hb and (b) deoxy-Hb represented in 10 minute epochs during absorption period and 9 minute epochs during cognitive task period following placebo and beetroot supplement. Means and SEM are presented as change from pre-treatment, resting baseline. Treatment x epoch interaction effects are shown. Significance is compared to placebo (t-tests calculated with the Mean Squares Error from the ANOVA) (* $p<0.05$, ** $p<0.01$, *** $p<0.005$, **** $p<0.001$).

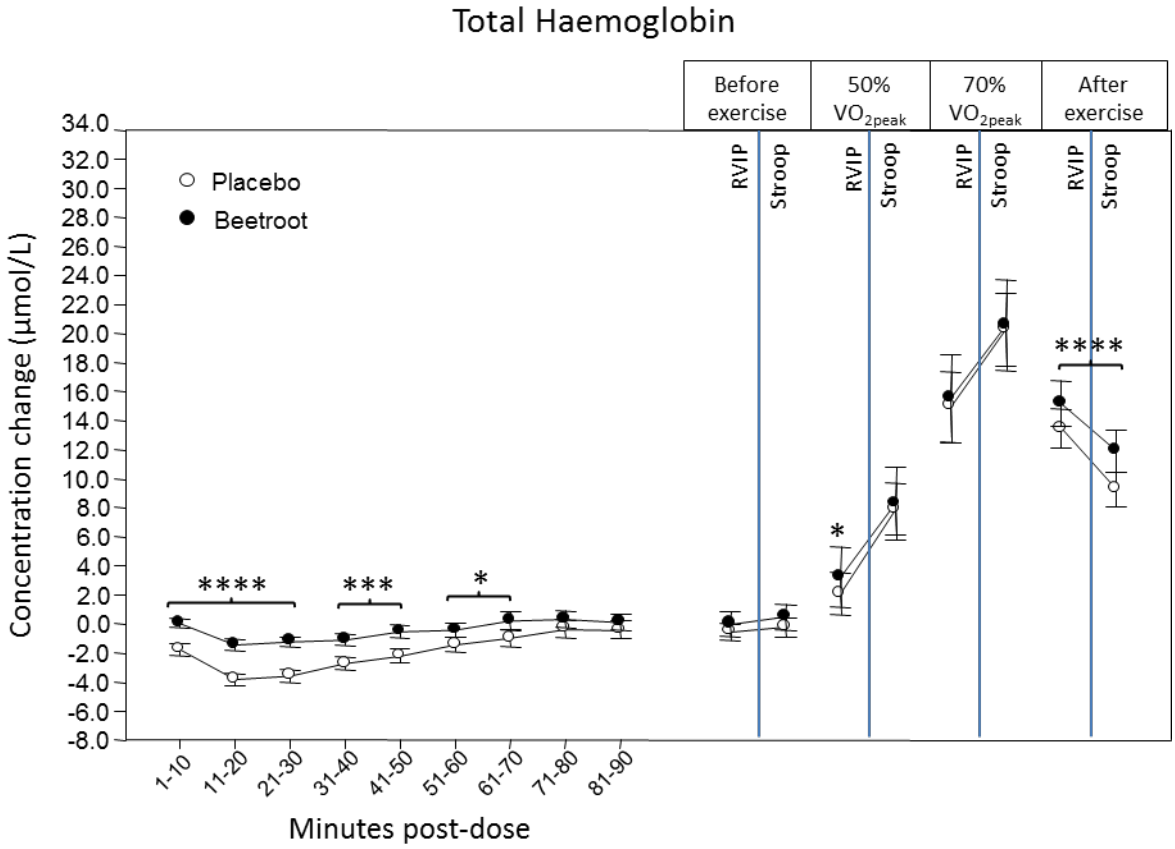


Fig. 6.5. Concentration change of total-Hb represented in 10 minute epochs during absorption period and 9 minute epochs during cognitive task period following placebo and beetroot supplement. Means and SEM are presented as change from pre-treatment, resting baseline. Treatment x epoch interaction effects are shown. Significance is compared to placebo (t-tests calculated with the Mean Squares Error from the ANOVA) (* $p<0.05$, *** $p<0.005$, **** $p<0.001$).

6.3.2.1.4 Further primary analysis

Effects of treatment on cerebral blood flow during performance of individual tasks.

6.3.2.1.4.1 Oxygenated haemoglobin

There were no treatment related differences specific to task performance for oxy-Hb.

6.3.2.1.4.2 Deoxygenated haemoglobin

There were no treatment related differences specific to task performance for deoxy-Hb.

6.3.2.1.4.3 Total haemoglobin

There were no treatment related differences specific to task performance for total-Hb.

6.3.2.2 Secondary analysis

Effects of treatment on cerebral blood flow during task performance using smaller duration (3 minute) epochs.

6.3.2.2.1 Oxygenated haemoglobin

There were no treatment related differences in task performance for oxy-Hb.

6.3.2.2.2 Deoxygenated haemoglobin

A significant interaction effect (treatment X task X exercise level X epoch) was observed for deoxy-Hb [$F(6, 90)=2.29$, $p<0.05$]. Planned comparisons revealed that deoxy-Hb was significantly reduced across both the RVIP and Stroop tasks, before exercise commenced (all $p<0.05$), during 50 % VO_{2peak} (all $p<0.005$), 70 % VO_{2peak} (all $p<0.05$) and following exercise completion (all $p<0.001$), see fig. 6.6 for full details of significance values.

6.3.2.2.3 Total haemoglobin

There were no treatment related differences in task performance for total-Hb.

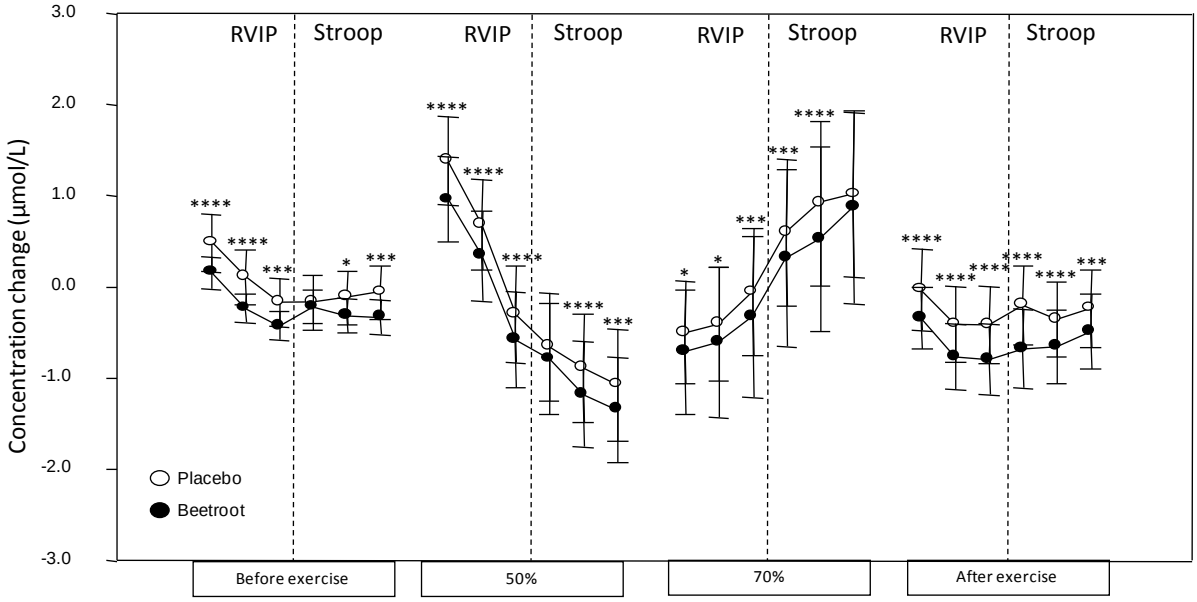


Fig. 6.6. Concentration change of deoxy-Hb during cognitive task periods, represented in 3 minute epochs following placebo and beetroot supplement. Means and SEM are presented as change from pre-treatment, resting baseline. Treatment x task x exercise level x epoch interaction effects are shown. Significance is compared to placebo (t-tests calculated with the Mean Squares Error from the ANOVA) (* $p<0.05$, *** $p<0.005$, **** $p<0.001$).

6.3.3 Cognitive performance and subjective fatigue

6.3.3.1 Baseline measures

Due to a data capture error, only 15 participants were included in the cognitive and subjective analysis. There were no significant on-day differences in cognitive performance, subjective mental fatigue or energy level measures prior to treatment.

6.3.3.2 Cognitive performance

There were no treatment related significant differences in performance on cognitive tasks.

6.3.3.3 Subjective mental fatigue and energy levels

There were no treatment related significant differences in subjective mental fatigue or energy level measures.

Table 6.1. Baseline and change from baseline scores for RVIP and Stroop tasks for each treatment. Means ±SEM values are presented with F and p values from the primary ANOVA of treatment effects and treatment x exercise level interactions. Significant measures are shown in bold.

Measure	*n*	Treat	Baseline	Post-dose change from baseline score				Treat effect	Treat x exercise level interaction
				Exercise Level					
				Before Exercise	50 % VO_{2peak}	70 % VO_{2peak}	After Exercise		
RVIP correct (%)	15	Placebo	59.17±4.44	-10.46±4.73	-14.26±3.40	-19.63±3.94	-12.41±5.34	F<1	F<1
		Beetroot	62.50±4.23	-10.93±3.62	-12.50±3.76	-23.61±4.33	-14.26±5.07		
RVIP RT (ms)	15	Placebo	505.8±13.14	4.84±12.91	0.435±12.36	-13.56±13.20	-23.52±13.63	F<1	F<1
		Beetroot	498.1±9.95	-5.71±8.60	-0.516±13.16	-5.75±12.26	-13.15±11.09		
RVIP false alarms (%)	15	Placebo	9.58±2.92	-1.77±2.35	-0.955±2.21	0.324±2.80	-2.73±2.66	F<1	F<1
		Beetroot	5.00±0.905	1.22±1.51	1.95±1.88	5.29±1.94	1.02±1.30		
Stroop overall correct (%)	15	Placebo	97.84±0.55	-0.317±0.46	-0.20±0.65	-0.41±0.64	0.128±0.73	F<1	F<1
		Beetroot	97.71±0.56	0.113±0.61	-0.33±0.61	-0.51±0.55	0.251±0.49		
Stroop overall RT (ms)	15	Placebo	623.1±24.10	2.13±12.41	29.27±10.60	183.5±109.9	10.13±20.33	F<1	F<1
		Beetroot	630.6±26.98	-2.93±17.94	29.93±27.54	139.8±70.89	-8.20±18.91		

Table 6.2. Baseline and change from baseline scores for subjective mental fatigue and energy level visual analogue scales. Means ±SEM values are presented with F and p values from the primary ANOVA of treatment effects and treatment x exercise level interactions. Significant measures are shown in bold.

Measure	*n*	Treat	Baseline	Post-dose change from baseline score					Treat effect	Treat x exercise level interaction
				Exercise Level						
				Before Exercise	50 % VO_{2peak}	70 % VO_{2peak}	90 % VO_{2peak}	After Exercise		
Mental Fatigue (mm)	15	Placebo	17.40±3.87	22.57±5.10	22.63±4.58	42.40±5.69	37.93±6.05	45.43±7.04	F<1	F<1
		Beetroot	23.57±4.63	15.80±2.73	23.97±3.54	39.17±5.13	40.50±4.38	41.70±6.20		
Energy Level (mm)	15	Placebo	39.20±4.81	-0.267±3.88	12.57±4.73	0.500±6.45	-14.73±7.65	-7.60±5.79	F<1	F<1
		Beetroot	41.33±6.01	2.67±4.19	11.17±4.22	-0.67±6.87	-18.10±7.83	-9.53±5.61		

6.4 Discussion

The initial findings of the study were that acute consumption of 500 ml BR led to a significant increase in plasma nitrite levels that were observed at 90 minutes and maintained at ~ 180 minutes post-dose. In terms of cerebral oxygenation, following BR supplementation there was a significant increase in oxy-Hb during the first 70 minutes of the absorption period that was followed by a significant increase during the RVIP task at 50 % VO_{2peak} and post-exercise during both the RVIP and Stroop tasks, these findings were largely mirrored for total-Hb. BR supplementation also led to a significant reduction in deoxy-Hb during performance of the RVIP and Stroop tasks pre and post exercise and during exercise at 50 and 70 % VO_{2peak}. Overall, BR supplementation significantly increased oxy-Hb and total-Hb and led to a significant decrease in deoxy-Hb.

There were no effects of BR supplementation on cognition or measures of subjective fatigue.

The demonstration that an acute 500 ml dose of BR leads to an elevation in plasma nitrite levels at 90 and 180 minutes post-consumption is a finding that has been documented previously and within similar time-scales (Kenjale et al., 2011; Vanhatalo et al., 2010; Webb et al., 2008; Wightman, Haskell-Ramsay, Thompson, et al., 2015). In relation to cerebral blood flow, a principal finding of the present study was that following BR supplementation during exercise and concomitant performance of cognitive tasks, cerebral haemodynamics were modulated. The initial change in oxy-Hb and total-Hb was observed during the absorption period within the first 10 minutes and lasted until approximately 70 minutes post-dose, when levels returned to those similar to the placebo treatment. That these effects should be observed so soon following treatment suggests it is unlikely that they are as a direct consequence of nitrate supplementation and its subsequent reduction to nitrite and NO. Previous research of the time course of dietary nitrate has observed that peak plasma concentrations of nitrate and nitrite have not been demonstrated until approximately 90 and 180 minutes post-dose, respectively (Webb et al., 2008). An alternative explanation for the augmentation seen following BR could be the sensory properties of the drink. Functional magnetic imaging research has

established that activation of the frontal cortex can occur as a direct result of the sensory properties of food and this has been demonstrated for different tastes (Kringelbach, de Araujo, & Rolls, 2004; Smits, Peeters, van Hecke, & Sunaert, 2007) as well as the valence of taste (its pleasant or unpleasantness) (Small et al., 2003). This is perhaps corroborated by the fact that after this initial elevation, and prior to the onset of the cognitive and exercise interventions, the difference between the treatments for oxy-Hb, deoxy-Hb and total-Hb no longer remains. In the present study, during the subsequent cognitive task period, the only treatment related significant increases in oxy-Hb and total-Hb that occurred were during the RVIP task at 50 % VO_{2peak} and once exercise had ceased. Masschelein et al. (2012) studied the haemodynamic effects of 500 ml dietary nitrate during exercise in hypoxia using NIRS and concluded that supplementation did not lead to any augmentation in cerebral oxygenation levels. However, studies that used TCD to measure CBF during exercise have reported significant reductions in cerebrovascular resistance index following 500 ml BR at 40 %, 60 % and 80 % $VO_{2\ peak}$ (Bond et al., 2013) and increases in MCA Vmean following a smaller BR dose (140ml) during exercise at 70 % and 85 % alongside simultaneous cognitive task (Stroop) performance (Rattray et al., 2015). Why nitrate should lead to an increase in oxy-Hb and total-Hb during the RVIP task in the initial stages of exercise, in the absence of any other exercise/task related effect, is therefore unclear. The only other effect of BR on oxy-Hb and total-Hb was observed during the recovery period, once exercise had ceased. In the absence of exercise intervention, BR supplementation has been shown to increase cerebral blood flow at rest in healthy older adults (Presley et al., 2011) and during performance of mentally demanding tasks in healthy young adults (Wightman, Haskell-Ramsay, Thompson, et al., 2015). The presence of an effect on oxy-Hb and total-Hb during the recovery period but not consistently prior to exercise performance may reflect the ability of BR/nitrate supplementation to modulate levels of total-Hb and oxy-Hb when it is needed most. With reference to the present study; when the participant is mentally and physically fatigued after a bout of intense exercise and cognitively demanding tasks.

Turning to the effects of BR on deoxy-Hb, although there was no change in deoxy-Hb during the absorption period, before, during and after exercise performance there was a significant reduction in deoxy-Hb. Recently a study by Aamand et al. (2013) identified that following dietary nitrate (in the form of sodium nitrate) a faster and smaller BOLD signal was observed, but only in response to visual stimuli. It was suggested that this reflected enhanced haemodynamic coupling as a result of increased nitrate intake. It is possible that in the present study, under similar circumstances (increased nitrate intake in the presence of a stimulus; with the stimulus in the current study being increasing exercise load and simultaneous performance of cognitive tasks), the same enhancement of haemodynamic coupling occurred. In the present study this was represented by a reduction in deoxy-Hb in the absence of a consistent increase in oxy-Hb across the cognitive task period as compared to placebo.

NIRS assessments of cerebral oxygenation during incremental exercise performance have observed that acute sub-maximal exercise leads to an increase in cerebral oxygenation in the PFC, but at maximal exercise oxy-Hb falls and deoxy-Hb increases (Rooks, Thom, McCully, & Dishman, 2010). In the present study, irrespective of treatment during initial task performance (before exercise had begun), oxy-Hb and total-Hb were at levels similar to the absorption period; however, once exercise commenced cerebral oxygenation rose incrementally with exercise level, before dropping off once exercise had ceased. Although the effects on deoxy-Hb began in the same manner, with levels during initial task performance similar to that of the absorption period, they then became more complex. During RVIP performance at 50 % VO_{2peak} there was an increase in deoxy-Hb followed by a reduction in deoxy-Hb during the second half of exercise performance (during Stroop) at 50 % VO_{2peak}, which was maintained during RVIP at 70 % VO_{2peak}, before peaking during Stroop at 70 % VO_{2peak} and then dropping to levels similar to the onset of task performance. This perhaps reflects a pattern of effects whereby the reduction in deoxy-Hb (as a consequence of the increase in oxy-Hb as task/exercise demands increase) is overridden when the demands of the task peak and resources are

required most, demonstrated by an increase in deoxy-Hb in the presence of an increase of oxy-Hb.

The current study failed to demonstrate an effect of BR supplementation on performance of cognitive tasks. The absence of an effect of BR supplementation on cognitive performance, specifically during RVIP and Stroop tasks which were used here, is one that has been observed previously in an older cohort (Kelly et al., 2013) as well as in healthy young populations (Rattray et al., 2015; Wightman, Haskell-Ramsay, Thompson, et al., 2015). It could be argued this may have been due to the sensitivity of the tasks and their ability to detect an effect in the present study during exercise. However, previous research of cognitive tasks in the presence of exercise has demonstrated that BR supplementation improved reaction time on the Stroop task during intermittent sprint performance (Thompson et al., 2015). A further explanation could have been the overly demanding nature of the protocol and the excessive equipment participants were required to wear (ICa face mask and NIRS head band), whilst simultaneously exercising and completing tasks.

There were some limitations to the present study. Although every attempt was made to blind participants to the drink they were receiving it is highly likely that they were able to discern a difference in taste and mouthfeel of the drinks. Further studies would benefit from better blinding of treatments, for example through the use of beetroot juice and placebo ‘shots’, which are currently available but were not at the time of testing. Although the integration of cognitive tasks and exercise performance was successful in the present study logistically, it may be that the method of delivery of the cognitive tasks and or the type of exercise intervention used, were not the most appropriate in order to obtain the most reliable results. This is perhaps reflected by the absence of any effects on cognitive performance.

Despite these limitations, the present study has demonstrated that NIRS is sensitive enough to detect changes in cerebral oxygenation during the performance of

cognitive tasks in the presence of incremental exercise and that BR supplementation via its conversion to nitrite can modulate these parameters.

Chapter 7. Discussion

7.1 Summary of the objectives of the thesis

The primary aim of this thesis was to identify and validate new approaches for the assessment of cognitive and physiological effects of nutritional interventions. This was achieved through the use of novel technologies and methodologies to expand upon existing knowledge of interventions and the methods by which these interventions can be measured. In order to address this, Chapter 2 investigated the behavioural and neurophysiological effects of ginkgo and a ginkgo/ginseng combination (herbal extracts with vasodilating properties). Here both electrophysiological and imaging techniques (EEG and NIRS) were used to measure cerebro-electrical and haemodynamic activity during the performance of a range of cognitive tasks. Chapter 3 explored the cognitive and cerebral oxygenation effects of ginkgo further by introducing a level of mental fatigue to the protocol. Chapter 4 aimed to identify the sensitivity of NIRS to assess the cognitive and cerebral haemodynamic effects of the vasoconstrictor caffeine in isolation and in combination with L-theanine. These two compounds are commonly consumed together, but thought to possess disparate behavioural and physiological properties when consumed alone versus in combination. Chapter 5 explored the impact of task difficulty on cerebral haemodynamics and whole body metabolism in the presence of two different doses of caffeine. Chapter 6 introduced an exercise paradigm and explored the effects of beetroot juice during incremental cycling on haemodynamics and behavioural performance of prolonged cognitive tasks.

7.2 General summary of the findings

The results of the studies making up this thesis have demonstrated that different technologies and methodologies can be implemented together successfully. They have also provided meaningful contributions to existing knowledge of the central and peripheral impact of specific nutritional interventions. Specifically, chapters 2, 3 and 4 demonstrated the sensitivity of NIRS in measuring cerebral oxygenation following the administration of

interventions with vasoconstrictive and vaso-relaxing properties. In particular, that NIRS is sensitive enough to identify the ability of one intervention to augment another (chapter 4). Chapter 5 established that NIRS can be measured simultaneously with ICa and that a similar pattern of haemodynamic and metabolic effects is evoked during the performance of cognitive tasks, irrespective of treatment administered. Finally, chapter 6 demonstrated that NIRS can be applied effectively during concurrent exercise and cognitive performance. As well as being described in tables 7.1, 7.2, 7.3 and 7.4, a discussion of these findings is also included below.

Table 7.1 Significant effects of treatment on oxy-Hb, deoxy-Hb and total-Hb during performance of cognitive tasks

Measure	Task	Treatment									
		180 mg Gink	360 mg Gink	207 mg Gink	207 mg Gink + 207 mg Gins	75 mg Caff (ch 4)	50 mg L-thea	75 mg Caff + 50 mg L-thea	75mg Caff (ch 5)	150 mg Caff	500 ml Beet
Oxy-Hb	SRT	□	□	□	□	⬇			□	□	□
	CRT	□	□	□	□	⬇			□	□	□
	Serial 3s	□	□								□
	Serial 7s										□
	Serial 13s			□	□	□	□	□	□	□	□
	Serial 17s			□	□	□	□	□			□
	Stroop	□	□			⬇			□	□	⬆⬆
	RVIP					⬇					⬆⬆
	3-Back					□	□	□			□
Deoxy-Hb	SRT	□	□	□	□	⬆			□	□	□
	CRT	□	□	□	□			⬆⬆	□	□	□
	Serial 3s	□	□			⬆⬆		⬆⬆			□
	Serial 7s	⬆⬆				⬆⬆		⬆⬆			□
	Serial 13s	⬆⬆	⬇⬇	□	□	□	□	□	□	□	□
	Serial 17s		⬇⬇	□	□	□	□	□			□
	Stroop	□	□	⬆⬆		⬆⬆			□	□	⬇⬇
	RVIP			⬆		⬆⬆		⬆			⬇⬇
	3-Back		⬇	⬆		□	□	□			□
Total-Hb	SRT	□	□	□	□				□	□	□
	CRT	□	□	□	□				□	□	□
	Serial 3s	□	□								□
	Serial 7s										□
	Serial 13s			□	□	□	□	□	□	□	□
	Serial 17s			□	□	□	□	□			□
	Stroop	□	□						□	□	⬆⬆
	RVIP										⬆⬆
	3-Back					□	□	□			□

□ = not tested, ↑/↓ = increase/decrease during some time points within task ⬆/⬇ increase/decrease throughout duration of task.

Table 7.2 Significant effects of treatment on performance of cognitive tasks										
Task	180 mg Gink	360 mg Gink	207 mg Gink	207 mg Gink + 207 mg Gins	75 mg Caff (ch 4)	50 mg L-thea	75 mg Caff + 50 mg L-thea	75 mg Caff (ch 5)	150 mg Caff	500 ml Beet
SRT										
CRT accuracy										
CRT					⬇					
Serial 3s correct										
Serial 3s errors										
Serial 7s correct										
Serial 7s errors										
Serial 13s correct										
Serial 13s errors	⬇									
Serial 17s Correct										
Serial 17s errors										
Stroop % correct					⬆	⬆				
Stroop RT										
RVIP % accuracy										
RVIP RT										
RVIP FA				⬆ (acute)						
3-Back % correct										
3-back RT										

□= not tested, ↑/↓ = increase/decrease during some time points within task ⬆/⬇ increase/decrease throughout duration of task.

Table 7.3 Significant effects of treatment on subjective mood measures											
Measure		180 mg Gink	360 mg Gink	207 mg Gink	207 mg Gink + 207 mg Gins	75 mg Caff (ch 4)	50 mg L-thea	75 mg Caff + 50 mg L-thea	75 mg Caff (ch 5)	150 mg Caff	500 ml Beet
Bond Lader	Alertness										
	Calmness										
	Contented										
Caffeine research visual analogue scales	Relaxed										
	Alert										
	Jittery										
	Tired					⬇					
	Tense										
	Headache										
	Overall mood					⬆					
	'Alertness'					⬆					
	'Tension'										
Mental fatigue									⬇	⬇	
Concentration											
Mental stamina									⬆		
Physical stamina									⬆		
Mentally tired											
Physically tired											
Energetic											

Table 7.4 Significant effects of treatment on blood pressure and heart rate									
	180 mg Ginkgo	360 mg Ginkgo	207 mg Ginkgo	207 mg Ginkgo + 207mg Ginseng	75 mg Caffeine (chapter 4)	50 mg L-thea	75 mg Caffeine + 50 mg L-thea	75 mg Caffeine (chapter 5)	150 mg Caffeine
Systolic BP							⬆		
Diastolic BP					⬆	⬆	⬆		
Heart Rate									

□= not tested, ↑/↓ = increase/decrease during some time points within task ⬆/⬇ increase/decrease throughout duration of task.

7.3 The use of NIRS in the assessment of haemodynamic change during cognitive performance in interventional studies

The ability of NIRS to identify changes in cerebral oxygenation as a result of nutritional challenge has been validated in the current thesis following a range of different interventions, each known for their contrasting effects on CBF. Chapter 2 established that a 207 mg dose of ginkgo attenuated the decrease in deoxy-Hb seen following placebo, during tasks that activate the pre-frontal cortex (Stroop, 3-back and to some extent, RVIP).

A similar pattern of effects was also observed in chapter 3, during performance of serial subtractions tasks (serial 7s and 13s) following a 180 mg but not a 360 mg dose of ginkgo. Following the 360 mg dose, deoxy-Hb was significantly reduced as compared to placebo. At the methodological level, this differential effect on deoxy-Hb is a good example of the sensitivity of this method to detect changes in the haemodynamic response as a consequence of different levels of the same active compound. Chapter 4 highlighted that NIRS was also capable of detecting oxygenation changes when one intervention is combined with another. A 75 mg dose of caffeine administered alone led to a significant reduction in oxy-Hb during simple (SRT, CRT) and complex frontal tasks (Stroop, RVIP) as well as a during a rest period. This effect was abolished when the same caffeine dose was combined with 50 mg L-theanine (levels comparable to 1-2 cups of tea); an effect made more compelling by the absence of any effects when a 50 mg dose of L-theanine was administered in isolation. The same study also demonstrated that the two caffeine containing treatments led to significant increases in deoxy-Hb (for the majority of tasks administered, with exceptions for each treatment, see table 7.1). As discussed in chapter 4, this increase in deoxy-Hb was potentially indicative of neuronal activation evidenced by caffeine's propensity for neurovascular uncoupling as observed in previous fMRI studies (Chen & Parrish, 2009a; Perthen et al., 2008). This is the first study to demonstrate such an effect using the technique of NIRS during performance of cognitive tasks. Chapter 4 also highlighted the ability of NIRS to detect variations within consumer groups as demonstrated by opposing effects of 75 mg on deoxy-Hb, irrespective of task, in habitual and non-habitual consumers. The findings of chapter 5, however, were not consistent with those of chapter 4 with regards to caffeine's effects on cerebral oxygenation. Counter to previous studies which have consistently reported a significant decrease in CBF following caffeine (Chen & Parrish, 2009a; Laurienti et al., 2003; Mathew & Wilson, 1991; Rack-Gomer et al., 2009), 75 mg caffeine led to a significant increase in total-Hb during specific time points within the absorption period (starting at 6 minutes post-dose and continuing intermittently until the end of the absorption period). This finding was particularly unexpected since the same dose in chapter 4 led to a significant decrease in

oxy-Hb, observed as early as 3 minutes post-dose. In addition to this, neither the 75 mg nor 150 mg caffeine dose led to a significant change in cerebral haemodynamics during task performance. Previous NIRS studies of cognitive performance in the presence of caffeine (including chapter 4 of this thesis) have demonstrated a significant decrease in oxy-Hb and or total-Hb in both the presence and absence of a corresponding increase in deoxy-Hb during performance of cognitive tasks (Heilbronner et al., 2015; Higashi et al., 2004; Kennedy & Haskell, 2011; Niioka & Sasaki, 2003). It is not abundantly clear why the present study failed to find an effect on CBF of these two doses, particularly since the doses in the aforementioned studies were either similar to or the same as those used here. One explanation is the decision in chapter 5 to use only habitual consumers since, in both the study by Kennedy and Haskell (2011) and in chapter 4 it was the non-habitual consumer group for whom total-Hb was decreased and deoxy-Hb was increased relative to placebo. However, this does not account for the increase observed in total-Hb during the absorption period following 75 mg caffeine. An alternative explanation could be that the 75 mg dose administered here was not enough to counteract the increase in CBF that is seen in habitual consumers following a period of caffeine withdrawal (Addicott et al., 2009; Field et al., 2003). However, when the findings in relation to placebo are considered, this also does not fully account for the differences observed.

The findings in relation to beetroot juice and its modulation of cerebral oxygenation go further to highlight the ability of NIRS to identify the effects of nutritional interventions, in this instance, one with vaso-relaxing properties. Chapter 6 demonstrated that a 500 ml dose of beetroot juice led to a significant increase in oxy-Hb and total-Hb during the absorption period (intermittently during the first 70 minutes) and during performance of tasks in both the presence (RVIP) and absence (RVIP and Stroop) of exercise. Irrespective of epoch, it also led to a significant increase in oxy-Hb and total-Hb overall. In terms of deoxy-Hb, beetroot caused a significant reduction during the entire task period (RVIP and Stroop) and before, during and after exercise at 50 and 70 % VO_{2peak}, as compared to placebo. There was also a significant reduction in deoxy-Hb overall, as compared to placebo, irrespective of epoch. As discussed in chapter 6, the increases

observed during the absorption period are most likely related to the sensory properties of the drink, therefore of most interest here are the haemodynamic effects during performance of cognitive tasks. Prior to and during exercise, the only effect observed in relation to oxy-Hb and total-Hb was a significant increase at 50 % VO_{2peak} during the RVIP task. Previous research of BR supplementation (doses equivalent to and less than those used here) has demonstrated that during exercise at 40, 60, 70, 80 and 85 % VO_{2peak}, cerebrovascular resistance index CRI is reduced and middle cerebral artery mean blood velocity MCA Vmean is elevated (Bond et al., 2013; Rattray et al., 2015), but only at 70 and 85 % VO_{2peak} during the simultaneous performance of the Stroop task (Rattray et al., 2015). The absence of any further changes in oxy-Hb or total-Hb in the present study during simultaneous exercise/cognitive task performance may therefore relate to the specifics of the methodology used, since in the study by Rattray et al. (2015) cycling was conducted for only 8 minutes with the Stroop task completed over the last 3 minutes of this exercise bout and not throughout. Based on previous research therefore, a protocol with a shorter bout of intense exercise and or a reduced cognitive workload may have been more successful in eliciting an effect of this measure. Interestingly once exercise had ceased oxy-Hb and total-Hb were significantly elevated once more during the RVIP task and also the Stroop task. This may reflect the ability of BR to augment cerebral oxygenation when it is needed most, in this case at the onset of simultaneous exercise and cognitive performance and then following extended periods of physical and mental fatigue. Turning to the effects on deoxy-Hb, here a consistent decrease during task completion prior to, during and after exercise following BR supplementation, may represent enhanced haemodynamic coupling (Aamand et al., 2013). It also demonstrates the capacity of the method adopted to detect modulation of cerebral haemodynamics in physiologically and cognitively demanding circumstances following nutritional intervention.

7.4 Integration of technologies in the assessment of nutritional interventions during cognitive challenge

One of the main aims of the current thesis was to identify technologies that could be used together in the assessment of nutritional interventions and their effects upon cognition. Chapter 2 aimed to do this through the integration of NIRS and EEG in the assessment of two herbal extracts that had previously been shown to modulate EEG. The intention being, that inclusion of NIRS would elucidate any haemodynamic effects of the extracts, whilst contributing to the understanding of the changes observed via EEG. Although EEG and NIRS were measured within the same study, it was not possible in the current thesis to integrate the two methods and simultaneously monitor both, despite this being the initial intention. This was primarily due to the equipment available not allowing both devices to be secured to the head at the same time, without impacting on the quality of the data.

Chapter 5, however, was more effective in this respect and the successful integration of NIRS with ICa led to some interesting findings, albeit irrespective of the treatment administered. The subjective difficulty scales participants completed following each task revealed that numerically, participants found the least difficult task the control, followed by serial 3s, serial 7s, serial 17s and 3-back with RVIP rated as the most difficult. However, despite this, the assessments of cerebral oxygenation and metabolism during task performance revealed a different pattern of effects to the subjective assessment and a similar pattern of effects to each other. The serial subtraction of 3s, 7s and 17s led to significantly greater increases in oxy-Hb and total-Hb compared to the control and correspondingly the smallest numerical reductions in deoxy-Hb. The 3-back and RVIP task, however, were at levels similar to that of the control. The same subtraction tasks (serial 3s, serial 7s and serial 17s) also led to significantly greater increases in energy expenditure and numerically greater carbohydrate oxidation, compared to control. Once again, the 3-back and RVIP tasks were at levels more comparable to that of the control task. Furthermore, the cognitive demands of the task were nicely demonstrated in the ICa data by comparing the metabolic effects of serial 3s to the somatically matched control.

These findings are a good example of how integrating technologies can provide a new insight into the physiological effects of task performance. They also demonstrate how simultaneous assessments can validate the observed effects of each measure.

7.5 Integration of methodologies in the assessment of nutritional interventions during cognitive performance

7.5.1 Manipulating cognitive demand/fatigue

Chapter 3 used cognitive tasks to manipulate the level of cognitive demand and by consequence the level of fatigue experienced by participants. This was achieved through the repeated administration of a block of tasks previously identified as being the most subjectively difficult within the COMPASS testing battery (Wightman, 2013). It was anticipated that this method would protect against any ceiling effects likely to occur. It would also highlight any behavioural and or physiological effects of the nutritional intervention (in this instance ginkgo) in the healthy young population being assessed. Unfortunately, however, no clear effects resulting from the manipulation of demand/fatigue were observed on behavioural performance. As discussed in chapter 3 this may be due to the tasks not being difficult or fatiguing enough to elicit an effect. However, this does not seem the most probable explanation since this approach (using similar but fewer tasks than those used in the aforementioned chapter), has previously been observed to elicit effects in the presence of a range of interventions (Kennedy & Scholey, 2004; Reay et al., 2005, 2006; Scholey et al., 2009). An alternative explanation is that repeated administration of this particular set of tasks was in fact found to be too demanding, leading participants to essentially ‘give up’ and thereby inadvertently removing the level of demand all together. It should be noted that the tasks followed a 90-minute absorption period during which participants were required to wear the NIRS headgear and restrict body movement. It seems, therefore, even more plausible that they may have found the entire testing period overly fatiguing and consequently disengaged in the tasks. With regards the absence of an effect on cerebral oxygenation, the purpose of the repeated use of demanding tasks was to create an environment whereby any potential increase in CBF would be more readily apparent. Unfortunately following ginkgo supplementation,

this was not the case. Although changes in deoxy-Hb were observed, they were not as a consequence of the level of demand but of the dose administered. The 180 mg led to an increase and the 360 mg dose lead to a decrease in deoxy-Hb overall during individual task performance, irrespective of task repetition. This finding may also be explained by an unwillingness to engage once consistent repetition of tasks became too demanding/fatiguing. Or, it may simply reflect that in young, healthy populations such as in the present study, CBF is not readily augmented as a consequence of demand. Chapter 5 aimed to measure the effects of cognitive demand in a different way, through the concomitant assessment of metabolism via ICa during performance of tasks with increasing ratings of subjective difficulty and mental fatigue. This study was more successful in its approach and demonstrated that measuring energy expenditure during the performance of cognitive tasks allowed not only the identification of those that were the most metabolically demanding, but also those that were the most cognitively demanding. Here, simultaneous measures of cerebral oxygenation (via NIRS), and metabolism (via ICa), identified the same serial subtraction tasks as requiring the most resources, both cognitively and physiologically. This study also highlighted that subjective ratings of the level of demand/difficulty of a task may not always be the most reliable method of assessment of this measure. Here the tasks rated as the least subjectively difficult led to increases in energy expenditure, oxy-Hb and total-Hb that were significantly greater than the control task and those that were identified as the most subjectively difficult (3-back and RVIP) led to changes in cerebral oxygenation and metabolism that were more in keeping with the control task. It may be that what defines the 'difficulty' or level of 'mental fatigue' of a cognitive task is much more complex than the ratings a subjective measure can convey.

7.5.2 Exercise, cognitive demand and fatigue

Chapter 6 introduced exercise to the protocol in an attempt to increase the demand/fatigue placed on participants during performance of cognitive tasks in the presence of BR juice. The foremost indication of the impact of exercise can be seen in figures 6.4 and 6.5 where the effects of incremental exercise are clearly demonstrated

across oxy-Hb, deoxy-Hb and total-Hb. Following placebo, prior to exercise during cognitive performance, both oxy-Hb and total-Hb are at levels comparable to those during the absorption period. However, once exercise begins, the levels increase in line with intensity of exercise, before dropping off once exercise has ceased. The effects on deoxy-Hb, however, are more complex. There is an initial increase in deoxy-Hb in response to exercise performance at 50 % VO_{2peak}, an effect which is potentially as a result of the concomitant increase seen in oxy-Hb, since there is an attenuation of the increase in deoxy-Hb during the second half of exercise performance at 50 % VO_{2peak} and the first half performance at 70 % $VO_{2peak.}$ This effect is subsequently overridden in the second half of performance at 70 % VO_{2peak} (during the Stroop task) where once again there is an increase in deoxy-Hb. This final increase in deoxy-Hb may be due to demand/fatigue being at its highest at this point – at the end of an intensive exercise bout and accompanying cognitive load. However, some of the most interesting findings of this chapter in relation to exercise, cognitive demand and fatigue, were from the effects on cerebral oxygenation in the presence of BR. Of most significance were those on deoxy-Hb, since there was a consistent pattern of significant effects across the entire cognitive testing period in both the presence and the absence of exercise. Here BR led to a significant decrease in deoxy-Hb as compared to placebo. This decrease was consistent and sustained throughout exercise at intensities of 50 % and 70 % VO_{2peak} during the RVIP and the Stroop tasks, as well as prior to and once exercise had ceased. As previously discussed, it is suggested that this is demonstrative of enhanced haemodynamic coupling in the presence of a stimulus as a result of nitrate supplementation (Aamand et al., 2013). That NIRS was capable of identifying an effect of BR supplementation in the presence of incremental exercise during the performance of cognitive tasks is particularly relevant here. It demonstrates the ability and sensitivity of this technique to detect exercise-related manipulation of demand/fatigue during cognitive performance in the presence of an intervention. Unfortunately, the present study revealed no effects of BR on behavioural performance in the presence of incremental exercise. However, the research to date pertaining to the effects of BR on performance of RVIP and

Stroop tasks administered here could be described as equivocal. Previous research has documented positive effects in the presence of exercise (Thompson et al., 2015) as well as an absence of effects both with (Rattray et al., 2015) and without exercise (Kelly et al., 2013; Wightman, Haskell-Ramsay, Thompson, et al., 2015).

7.6 Cognitive and mood effects of nutritional interventions

Ginkgo is a herbal extract that has led to a number of improvements in behavioural performance. Those that are of relevance to findings in the present thesis include sustained attention, speed of performance on attentional tasks, reduction in errors in performance of a serial 3s task, an increase in the number of correct responses made on a serial 7s task and improvements in feelings of alertness and contentment (Elsabagh et al., 2005; Kennedy et al., 2000, 2002). However, the only behavioural effects of ginkgo in isolation in the current thesis, were observed in chapter 3 following a 180 mg dose, where administration led to a reduction in the number of serial 13s errors. This is despite 360 mg (the same dose as administered in chapter 3) having been identified from a series of previous ginkgo assessments as the most beneficial in terms of its acute effects on cognition and mood in healthy young populations (Kennedy et al., 2002). One potential explanation for the limited findings is the short duration of the absorption period in chapter 3 and therefore the times at which testing took place. Previous ginkgo research identified that despite initial testing taking place 1-hour post-dose, the first behavioural effects in terms of speed of attention and mental arithmetic were observed at 2.5 hours and were still apparent at 4 and 6 hours post-dose (Kennedy et al., 2000, 2002). Practical limitations with continuous wave NIRS mean that removing the headband during testing is not possible. Consequently, the duration of a testing session must be carefully considered to avoid discomfort to participants. As previously discussed, the relative absence of effects in chapter 3 may also have been due to the increased cognitive demand placed on the participants, thereby masking any effects of treatment. Unfortunately, the explanation of demand does not extend to chapter 2 where a 207 mg ginkgo dose also failed to modulate behavioural performance as there was no extra

demand placed on the participant as a result of repeated administration of tasks, since they were only required to complete the tasks once. However, the explanation in terms of timing of post-dose session may be of more relevance since here, although testing began at 2.5 hours it only continued until just under 3 hours post-dose.

In chapter 2, 207 mg ginkgo was combined with 207 mg ginseng and this combination led to a significant increase in the number of RVIP false alarms following an acute dose. This was the first study to assess the behavioural effects of a combination of ginkgo and ginseng at these doses. The absence of any further findings than those reported here may be a reflection of the ratio of extracts used, and/or the comparatively low doses to previous research. Cognitive effects of this combination have been reported following doses of 120 mg ginkgo/200 mg ginseng, 240 mg ginkgo/400 mg ginseng, 360 mg ginkgo/600 mg ginseng (Kennedy et al., 2001a, 2002); in each case the ratio used has been more in favour of ginseng as opposed to ginkgo. It may also arise from the timings of post-dose testing since the majority of behavioural effects have been reported at 4 or 6 hours post-dose, with findings reported 1 and 2.5 hour(s) post-dose but only following the highest 960 mg combination (Kennedy et al., 2001a, 2002).

In chapters 4 and 5 the administration of caffeine in isolation at doses of 75 mg and (in chapter 5) 150 mg, led to fewer effects than perhaps expected given the known positive effects of caffeine on reaction time and vigilance (Childs & de Wit, 2006; Haskell et al., 2008b; Haskell et al., 2005; Quinlan et al., 2000; Rogers et al., 2008; Smit & Rogers, 2000). These effects have been observed across a range of studies and at similar doses and (in some instances) during the same tasks as those administered in chapters 4 and 5. Here, however, caffeine only led to a significant reduction in choice reaction time (largely as a result non-habitual caffeine consumers) and significantly improved performance accuracy on the Stroop task. Both effects were observed in chapter 4 and as a consequence of the 75 mg dose. That caffeine should lead to a reduction in reaction time and improve performance on what is largely an attentional task would therefore be as expected. As discussed previously, the absence of treatment effects on cognition in

chapter 5 is not entirely unexpected as tasks were selected based upon their potential to induce differing levels of metabolic demand, rather than their sensitivity to caffeine. Therefore, the absence of an effect during specific tasks previously sensitive to caffeine, may have been due to the demanding paradigm employed. In terms of the effects on mood, once again it was the 75 mg dose that led to the most prevalent effects; however, there were differences across the two studies despite the same dose and the same subjective assessment of mood being used in each. In chapter 4 the 75 mg dose led to a significant reduction in subjective ratings of tiredness and a significant increase in overall mood and ratings on the alertness factor. These subjective effects were not observed in chapter 5, even though the same 75 mg dose and caffeine research analogue scales were administered. However, the absence of an effect in terms of alertness may relate to the small sample size for this measure. In this chapter, the 75 mg and 150 mg dose of caffeine led to a significant reduction in subjective ratings of mental fatigue, with the 75 mg dose also leading to a significant increase in subjective ratings of mental and physical stamina. Once again that doses of 75 mg and 150 mg should lead to improvements in mood is consistent with previous caffeine research.

Chapter 4 also assessed the cognitive effects of a 50 mg dose of L-theanine. At this dose L-theanine led to a significant increase in performance accuracy on the Stroop task that was irrespective of caffeine consumer status. Previous L-theanine research has tended to report decrements or an absence in effects on behavioural performance, therefore this study is the first to report a positive effect on performance of the Stroop task. This may be due to the lower dose used here, as previous studies have tended to use doses that are much higher, of between 100 mg and 250 mg. There was also an effect of the combination of these two interventions. Studies that have assessed the combination of caffeine and L-theanine have observed improvements on a number of tasks that were not seen when each treatment was administered in isolation. Here 75 mg caffeine and 50 mg L-theanine together led only to a significant decrease in choice reaction time, an effect that was dependent upon consumer status since it was only observed in the non-habitual caffeine consumer group. Previous research has documented positive effects of this

combination on the same tasks as administered in this chapter (such as SRT, RVIP, alertness, tiredness, mental fatigue and headache ratings), meaning the relative paucity of effects observed here was unexpected. However, the majority of these studies used higher does and ratios more in favour of L-theanine than caffeine and this may explain the comparative lack of effects seen here as a result of this combination.

In chapter 6 the behavioural effects of 500 ml of BR were measured. In this study, however, no significant effects were observed on cognitive performance in the presence or absence of exercise and no effects on measures of subjective fatigue. Positive effects on cognitive performance during exercise such as those in the aforementioned study have only been demonstrated in one study to date (Thompson et al., 2015), other assessments of BR have reported no effects of cognitive performance in the presence (Rattray et al., 2015) as well as the absence of exercise (Kelly et al., 2013; Wightman, Haskell-Ramsay, Thompson, et al., 2015).

Cognitive and mood effects have been demonstrated in the present thesis as a result of the majority of the interventions assessed. It could be argued, however, that the number of findings are relatively modest for interventions that have previously reported relatively robust behavioural effects, in particular caffeine. This may be due in part to the relatively demanding nature of each protocol in that most required participants to remain seated for a lengthy period of time, during long absorption and cognitive tasks periods, wearing head gear that may have begun to feel uncomfortable. These factors may also have contributed to the general paucity of effects observed.

7.7 Concomitant changes in physiological and behavioural parameters following nutritional intervention

One of the main aims of the current thesis was integration of technologies and/or methodologies in the presence of nutritional interventions. The purpose of this aim was to identify cognitive and physiological benefits and assess the ‘bigger picture’ in relation to the means by which these effects may occur. There are 3 instances within this thesis

where a positive effect on behaviour is associated with a concomitant modulation of cerebral oxygenation in the presence of a nutritional intervention. In chapter 3, the significant reduction in the number of false alarms during the serial 13s subtraction task occurred in the presence of a significant increase in deoxy-Hb. Previous observations of increases in deoxy-Hb (in the presence of an increase in oxy-Hb) following interventions known to increase CBF have led authors to suggest that this may be indicative of increased oxygen extraction and utilisation (Bonoczk et al., 2002; Kennedy, Wightman, et al., 2010). It is possible that here, the increase in deoxy-Hb following the 180 mg dose of ginkgo also reflects an increase in oxygen extraction (in the absence of oxy-Hb), leading to the improvement in task performance seen. The two further concomitant changes in cerebral oxygenation and behaviour occurred in chapter 4 as a result of the 75 mg caffeine dose. In this study a significant reduction in CRT and improvement in Stroop performance accuracy were observed alongside significant reductions in oxy-Hb, with a concomitant increase in deoxy-Hb during performance of the Stroop task only. The increase in deoxy-Hb observed during the Stroop task in the presence of significantly reduced oxy-Hb, is reflective of caffeine's known ability to uncouple the relationship between CBF and oxygen consumption, by reducing blood flow but increasing oxygen consumption in response to task stimulation (Chen & Parrish, 2009a; Perthen et al., 2008). Once again the findings in relation to the Stroop task in particular may be indicative of increased oxygen extraction, which in the current context has translated as an improvement in Stroop performance. Unfortunately, there were no further associated effects on cerebral oxygenation and behavioural parameters. Changes in cerebral oxygenation were frequently observed in the absence of any change in behavioural performance and vice versa (although this was much less common).

7.8 Potential methodological limitations

Despite the novel and interesting findings observed as a consequence of the studies that make up this thesis, there were a number of methodological limitations that should be addressed.

Chapter 2 presented the findings from the initial NIRS study within this thesis; however here, unfortunately, no baseline NIRS measure was made in the absence of treatment, an omission that also extends to the cognitive performance measures. The CW-NIR technology used in the current thesis is one whereby the readings obtained are not absolute values, but are concentration change values. In functional studies that include an intervention, the baseline measure is generally in the absence of treatment (Kennedy & Haskell, 2011; Kennedy et al., 2016; Kennedy, Wightman, et al., 2010; Wightman, Haskell-Ramsay, Reay, et al., 2015; Wightman, Haskell-Ramsay, Thompson, et al., 2015; Wightman et al., 2012). However, the baseline data obtained in this study was after treatment had been administered. Therefore, any early effects of treatment may have already occurred, potentially diluting any treatment related findings as a consequence. There is also the possibility that they were missed altogether. With regards to the cognitive performance data, on-day differences in cognitive performance were not accounted for either in chapter 2. This was rectified in chapter 3 and the remaining 3 experimental chapters where baseline measures of cerebral oxygenation and cognitive performance in the absence of treatment were taken.

The methodological approach of using the continuous repetition of cognitive tasks and incremental exercise in order to increase the level of demand experienced by the participants was adopted in chapters 2 (ginkgo) and 6 (beetroot) respectively. Unfortunately, however, neither paradigm was successful in prompting an effect of treatment on cognitive performance. It is possible that the treatments administered and the doses selected were simply not capable of eliciting an effect. However, it is also possible that the demand required of the participants was too high. The use of the CDB within interventional studies has demonstrated how this approach (i.e. the use of demand) can be successful (Kennedy & Scholey, 2004; Reay et al., 2005, 2006; Scholey et al., 2009). Although the number and type of tasks used in chapter 3 was based upon the CDB, a more demanding schedule was presented. This was achieved through the use of more difficult versions of serial subtractions (7s, 13s and 17s as compared to 3s and 7s). Plus, the addition of 2 further tasks to each ‘block’ (albeit leading to completion of each

block within a very similar time frame of 10 minutes here, as compared to 9 minutes for CDB). This increase in difficulty, coupled with the extensive duration of each study visit, in addition to the equipment participants were required to wear, may have been overly demanding and participants may have, in effect, given up. One method of addressing this in the future would be to marginally reduce the level of demand experienced by limiting the number of tasks and/or the number of times participants are required to repeat those tasks. Another approach would be to use interventions with shorter absorption periods to avoid any additional means of tiring participants.

The completion of baseline cognitive tasks immediately prior to the pre-treatment, resting NIRS baseline measure is an approach adopted throughout the thesis from chapter 3 onwards. Following analysis of chapter 3, the carryover effects of cognitive performance on cerebral oxygenation were controlled for by using the last 5 minutes of the 10-minute baseline reading (chapters 5 and 6) or including a rest period prior to the baseline measure (chapter 4). However, the time taken for CBF to return to resting values after a cognitive challenge is not one that can be readily determined when taking individual differences into account. This coupled with the fact that CW-NIRS does not provide absolute quantifiable values, means there is no clear indication when oxygenation parameters have returned to values that reflect rest. Therefore, future studies may benefit from measuring pre-dose, baseline NIRS values at the beginning of the study session (after a seated rest), rather than post-task, to avoid any carry over effects of those tasks.

7.9 Future research

Research into the methods by which nutritional interventions can be assessed would benefit from addressing some of the methodological limitations as identified previously as well as considering other issues in future research.

One of the most interesting findings within the current thesis was the concomitant effects observed on cerebral oxygenation and metabolism (energy expenditure) when NIRS was measured alongside ICa during cognitive task performance (chapter 5). It is

unclear why this effect occurred in the absence of treatment when caffeine is known to impact both CBF and metabolism. However, future research would benefit from using this protocol to assess the impact of other interventions, particularly those known to increase CBF. Furthermore, with the identification that ICa measures detect not only the somatic effects of a task but also those of a cognitive nature, the inclusion of an element of demand to the protocol would also be of interest. This could be achieved through the repeated administration of tasks (taking into consideration factors described within methodological limitations) in order to determine the impact upon metabolism.

The finding from chapter 4 that a 50 mg dose of L-theanine administered in combination with 75 mg dose caffeine attenuated the oxy-Hb effects of 75 mg caffeine alone, also requires further investigation. At the end of testing the effect of this combination could still be seen on oxy-Hb and future research should be aimed at determining how long this is sustained for. This is particularly relevant since modulation of CBF as a consequence of caffeine has been seen as long as 90 minutes post dose (albeit following a larger, 250 mg dose than administered here) (Mathew & Wilson, 1985). It would also be pertinent to observe the impact of different doses. Previous research has documented a synergistic effect of L-theanine and caffeine on cognitive performance at higher doses and at ratios more in favour of L-theanine to that which was administered in the present study. It would therefore be of interest to determine if the effects of this increased dose/ratio also extend to cerebral haemodynamics.

7.10 General Conclusions

The aims of this thesis were 3-fold, the first being the identification of technologies that could be used alone or together in the novel assessment of nutritional interventions and their effects upon cognition. Each chapter addressed this aim in its own way, with the common theme throughout each being the application of the technique of NIRS (in the presence or absence of another technology). Chapters 2-6 demonstrated that NIRS is sensitive enough to detect changes in cerebral oxygenation following administration of

nutritional interventions known for their differing effects on CBF. Chapters 2 and 3 of this thesis were the first to use this technology to measure the acute effects of ginkgo on CBF and observe differences in the effects on deoxy-Hb as a function of dose. Chapter 2 was also the first study to demonstrate that the chronic administration of a ginkgo and ginseng combination leads to modulation of cerebro-electrical activity. Chapter 4 represented the first study to utilise NIRS in the assessment of L-theanine and an L-theanine and caffeine combination (interventions identified as having synergistic effects on cognitive performance when administered together). This study demonstrated that there are also differences in cerebral oxygenation when these two interventions are administered alone and in combination. The simultaneous measurement of NIRS and ICa in chapter 5 to identify the haemodynamic and metabolic impact of tasks of differing subjective difficulty, successfully demonstrated how two technologies can be effectively used together. Not only did this allow an assessment of the 'bigger picture', it also provided a more comprehensive understanding of an outcome as compared to one measure alone. Irrespective of treatment, the findings in relation to oxy-Hb and total-Hb were confirmed by those on energy expenditure. Here, the same tasks (serial subtractions) lead to increases across measures (NIRS and ICa) and a relative absence of an effect (when compared to the control task) during 3-back and RVIP across measures. Furthermore, the tasks requiring the most resources were not those with the highest subjective difficulty rating, leading to the conclusion that subjective measures of difficulty may not always be that accurate in identifying the level of peripheral and central resources required of a task. Chapter 5 also revealed that the cognitive demands as well as the somatic demands of a task are measured via ICa since energy expenditure was significantly elevated during serial 3s subtractions as compared to the control task, despite being somatically matched.

The second aim was to identify methodologies that could be implemented concomitantly during the assessment of interventions of interest to cognition. Unfortunately, in chapter 2 the approach of using demand as a tool (in the presence ginkgo) to provide an opportunity to enhance performance (or protect against decrements in performance) was unsuccessful. This may have been due to an overly demanding

protocol and future studies may be more successful in using a less demanding cognitive approach, as this method has proved effective previously. The use of exercise as a means of increasing demand led to more positive findings, however. In chapter 6, cerebral haemodynamics were augmented as a result of incremental exercise and the administration of beetroot juice led to a significant reduction in deoxy-Hb before during and after exercise performance. However, there were no behavioural effects as a consequence of increased demand in the presence of BR supplementation.

The third aim of this thesis was to expand the current knowledge base on the ability of specific nutritional supplements to benefit cognitive and physiological performance. The findings of chapter 2 and 3 revealed that the haemodynamic effects of ginkgo administration may be dependent upon dose. Acute administration of a ~200 mg and 180 mg dose of ginkgo led to a significant attenuation of the decrease in deoxy-Hb seen following placebo during the performance of tasks that activate the pre-frontal cortex. However, a 360 mg dose led to an opposing effect, whereby deoxy-Hb was significantly reduced. This is the first study to document an effect of dose on cerebral haemodynamics following acute ginkgo administration. In chapter 4, the significant reduction in oxy-Hb observed during task performance following 75 mg caffeine, was abolished when it was administered in combination with 50 mg L-theanine (doses equivalent to 1-2 cups of tea). This was despite no effects on this measure when L-theanine was administered in isolation. This finding is a similar pattern of effects to those observed on blood pressure (Rogers et al., 2008) and contributes to caffeine/L-theanine research of a synergistic effect between these two interventions. Chapter 6 demonstrated that BR supplementation can modulate cerebral haemodynamics during cognitive performance in the presence and absence of incremental exercise. Here BR led to a significant reduction in deoxy-Hb as compared to placebo during performance of all cognitive tasks and a significant increase in oxy-Hb and total-Hb at the beginning of exercise and following exercise completion.

The main findings of this thesis are therefore:

- That NIRS is sensitive enough to detect changes in cerebral oxygenation as a result of nutritional challenge; following differing doses of the same intervention; as a result of the synergistic effect of two different interventions and during incremental exercise whilst performing cognitive tasks.
- The identification that NIRS and ICa can be measured concomitantly and are effective in simultaneously determining the somatic and cogntive demands of a task, irrespective of treatment.
- That that this is the first study to demonstrate that caffeine administration leads to an increase in energy expenditure and carbohydrate oxidation during cogntive performance.
- That L-theanine administered at a dose comparable to 1-2 cups of tea antagonises the effects of caffeine in isolation on oxy-Hb.

References

Aamand, R., Dalsgaard, T., Ho, Y.-C. L., Moller, A., Roepstorff, A., & Lund, T. E. (2013). A NO way to BOLD?: Dietary nitrate alters the hemodynamic response to visual stimulation. *Neuroimage, 83*, 397-407. doi: 10.1016/j.neuroimage.2013.06.069

Acheson, K. J., Gremaud, G., Meirim, I., Montigon, F., Krebs, Y., Fay, L. B., . . . Tappy, L. (2004). Metabolic effects of caffeine in humans: lipid oxidation or futile cycling? *American Journal of Clinical Nutrition, 79*(1), 40-46.

Acheson, K. J., Zahorskamarkiewicz, B., Pittet, P., Anantharaman, K., & Jequier, E. (1980). Caffeine And Coffee - Their Influence On Metabolic-Rate And Substrate Utilization In Normal Weight And Obese Individuals. *American Journal of Clinical Nutrition, 33*(5), 989-997.

Addicott, M. A., Yang, L. L., Peiffer, A. M., Burnett, L. R., Burdette, J. H., Chen, M. Y., . . . Laurienti, P. J. (2009). The Effect of Daily Caffeine Use on Cerebral Blood Flow: How Much Caffeine can we Tolerate? *Human brain mapping, 30*(10), 3102-3114. doi: 10.1002/hbm.20732

Ahlemeyer, B., & Krieglstein, J. (2003). Neuroprotective effects of Ginkgo biloba extract. *Cellular and Molecular Life Sciences, 60*(9), 1779-1792. doi: 10.1007/s00018-003-2185-x

Arcelin, R., Delignieres, D., & Brisswalter, J. (1998). Selective effects of physical exercise on choice reaction processes. *Perceptual and motor skills, 87*(1), 175-185.

Arciero, P. J., Gardner, A. W., Callesescandon, J., Benowitz, N. L., & Poehlman, E. T. (1995). Effects Of Caffeine Ingestion On Ne Kinetics, Fat Oxidation, And Energy-Expenditure In Younger And Older Men. *American Journal of Physiology-Endocrinology and Metabolism, 268*(6), E1192-E1198.

Astrup, A., Toubro, S., Cannon, S., Hein, P., Breum, L., & Madsen, J. (1990). Caffeine - A Double-Blind, Placebo-Controlled Study Of Its Thermogenic, Metabolic, And Cardiovascular Effects In Healthy-Volunteers. *American Journal of Clinical Nutrition, 51*(5), 759-767.

Bailey, S. J., Fulford, J., Vanhatalo, A., Winyard, P. G., Blackwell, J. R., DiMenna, F. J., . . . Jones, A. M. (2010). Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *Journal of Applied Physiology, 109*(1), 135-148. doi: 10.1152/japplphysiol.00046.2010

Bailey, S. J., Winyard, P., Vanhatalo, A., Blackwell, J. R., DiMenna, F. J., Wilkerson, D. P., . . . Jones, A. M. (2009). Dietary nitrate supplementation reduces the O-2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *Journal of Applied Physiology, 107*(4), 1144-1155. doi: 10.1152/japplphysiol.00722.2009

Bender, V., & McGlynn, G. (1976). The effect of various levels of strenuous to exhaustive exercise on reaction time. *European journal of applied physiology and occupational physiology, 35*(2), 95-101.

BioSemi. (2016). Headcaps. Retrieved 19.12.2016, 2016, from http://www.biosemi.com/headcap.htm

Boas, D. A., Dale, A. M., & Franceschini, M. A. (2004). Diffuse optical imaging of brain activation: approaches to optimizing image sensitivity, resolution, and accuracy. *Neuroimage, 23*, S275-S288.

Boas, D. A., Elwell, C. E., Ferrari, M., & Taga, G. (2014). Twenty years of functional near-infrared spectroscopy: introduction for the special issue. *Neuroimage, 85*, 1-5.

Bond, A., & Lader, M. (1974). The use of analogue scales in rating subjective feelings. *British Journal of Medical Psychology, 47*(3), 211-218.

Bond, H., Morton, L., & Braakhuis, A. J. (2012). Dietary nitrate supplementation improves rowing performance in well-trained rowers.

Bond, V., Curry, B. H., Adams, R. G., Asadi, M. S., Millis, R. M., & Haddad, G. E. (2013). Effects of Dietary Nitrates on Systemic and Cerebrovascular Hemodynamics. *Cardiology research and practice, 2013*.

Bonoczk, P., Panczel, G., & Nagy, Z. (2002). Vinpocetine increases cerebral blood flow and oxygenation in stroke patients: a near infrared spectroscopy and transcranial Doppler study. *Eur J Ultrasound, 15*(1-2), 85-91.

Breese, B. C., McNarry, M. A., Marwood, S., Blackwell, J. R., Bailey, S. J., & Jones, A. M. (2013). Beetroot juice supplementation speeds O2 uptake kinetics and improves exercise tolerance during severe-intensity exercise initiated from an elevated metabolic rate. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 305*(12), R1441-R1450.

Bridi, R., Crossetti, F. P., Steffen, V. M., & Henriques, A. T. (2001). The antioxidant activity of standardized extract of Ginkgo biloba (EGb 761) in rats. *Phytotherapy Research, 15*(5), 449-451. doi: 10.1002/ptr.814

Brisswalter, J., Arcelin, R., Audiffren, M., & Delignieres, D. (1997). Influence of physical exercise on simple reaction time: effect of physical fitness. *Perceptual and motor skills, 85*(3), 1019-1027.

Brisswalter, J., Collardeau, M., & Rene, A. (2002). Effects of acute physical exercise characteristics on cognitive performance. *Sports Medicine, 32*(9), 555-566.

Brown, L. A., & Riby, L. M. (2013). Glucose enhancement of event-related potentials associated with episodic memory and attention. *Food & function, 4*(5), 770-776.

Buckner, R. L., Bandettini, P. A., Oâ€™Craven, K. M., Savoy, R. L., Petersen, S. E., Raichle, M. E., & Rosen, B. R. (1996). Detection of cortical activation during averaged single trials of a cognitive task using functional magnetic resonance imaging. *Proceedings of the National Academy of Sciences, 93*(25), 14878-14883.

Bunce, S. C., Izzetoglu, M., Izzetoglu, K., Onaral, B., & Pourrezaei, K. (2006). Functional Near-Infrared Spectroscopy: An Emerging Neuroimaging Modality. *IEEE Engineering in Medicine and Biology Magazine,* (July/August), 54-62.

Cabeza, R., & Nyberg, L. (2000). Imaging cognition II: An empirical review of 275 PET and fMRI studies. *Journal of Cognitive Neuroscience, 12*(1), 1-47. doi: 10.1162/08989290051137585

Chance, B., Anday, E., Nioka, S., Zhou, S., Hong, L., Worden, K., . . . Thomas, R. (1998). A novel method for fast imaging of brain function, non-invasively, with light. *Optics Express, 2*(10), 411-423.

Chen, Y. F., & Parrish, T. B. (2009a). Caffeine's effects on cerebrovascular reactivity and coupling between cerebral blood flow and oxygen metabolism. *Neuroimage, 44*(3), 647-652. doi: 10.1016/j.neuroimage.2008.09.057

Chen, Y. F., & Parrish, T. B. (2009b). Caffeine dose effect on activation-induced BOLD and CBF responses. *Neuroimage, 46*(3), 577-583. doi: 10.1016/j.neuroimage.2009.03.012

Childs, E., & de Wit, H. (2006). Subjective, behavioral, and physiological effects of acute caffeine in light, nondependent caffeine users. *Psychopharmacology, 185*(4), 514-523. doi: 10.1007/s00213-006-0341-3

Chmura, J., Krysztofiak, H., Ziemba, A., Nazar, K., & Kaciuba-Uscilko, H. (1997). Psychomotor performance during prolonged exercise above and below the blood lactate threshold. *European journal of applied physiology and occupational physiology, 77*(1-2), 77-80.

Cho, H. S., Kim, S., Lee, S. Y., Park, J. A., Kim, S. J., & Chun, H. S. (2008). Protective effect of the green tea component, L-theanine on environmental toxins-induced neuronal cell death. *Neurotoxicology, 29*(4), 656-662. doi: 10.1016/j.neuro.2008.03.004

Clifford, T., Bell, O., West, D. J., Howatson, G., & Stevenson, E. J. (2016). The effects of beetroot juice supplementation on indices of muscle damage following eccentric exercise. *European journal of applied physiology, 116*(2), 353-362.

Cohen, J. D., Perlstein, W. M., Braver, T. S., Nystrom, L. E., Noll, D. C., Jonides, J., & Smith, E. E. (1997). Temporal dynamics of brain activation during a working memory task. *Nature, 386*(6625), 604-608. doi: 10.1038/386604a0

Collardeau, M., & Brisswalter, J. (2001). Effects of a prolonged run on simple reaction time of well trained runners. *Perceptual and motor skills, 93*(3), 679-689.

Collardeau, M., Brisswalter, J., Vercruyssen, F., Audiffren, M., & Goubault, C. (2001). Single and choice reaction time during prolonged exercise in trained subjects:

influence of carbohydrate availability. *European journal of applied physiology, 86*(2), 150-156.

Costill, D. L., Dalsky, G. P., & Fink, W. J. (1978). Effects Of Caffeine Ingestion On Metabolism And Exercise Performance. *Medicine and Science in Sports and Exercise, 10*(3), 155-158.

Coull, J. T., Frith, C. D., Frackowiak, R. S. J., & Grasby, P. M. (1996). A fronto-parietal network for rapid visual information processing: A PET study of sustained attention and working memory. *Neuropsychologia, 34*(11), 1085-1095. doi: 10.1016/0028-3932(96)00029-2

Craik, F. I., & Salthouse, T. A. (2008). *The handbook of aging and cognition*: Psychology Press.

Cui, X., Bray, S., Bryant, D. M., Glover, G. H., & Reiss, A. L. (2011). A quantitative comparison of NIRS and fMRI across multiple cognitive tasks. *Neuroimage, 54*(4), 2808-2821.

Cutini, S., Scatturin, P., Basso Moro, S., & Zorzi, M. (2014). Are the neural correlates of subitizing and estimation dissociable? An fNIRS investigation. *Neuroimage, 85*, 391-399.

Dangelo, L., Grimaldi, R., Caravaggi, M., Marcoli, M., Perucca, E., Lecchini, S., . . . Crema, A. (1986). A double-blind, placebo-controlled clinical-study on the effect of a standardized ginseng extract on psychomotor performance in healthy volunteers. *Journal of ethnopharmacology, 16*(1), 15-22. doi: 10.1016/0378-8741(86)90063-2

Davis, J. M., Zhao, Z. W., Stock, H. S., Mehl, K. A., Buggy, J., & Hand, G. A. (2003). Central nervous system effects of caffeine and adenosine on fatigue. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 284*(2), R399-R404. doi: 10.1152/ajpregu.00386.2002

Davranche, K., & Audiffren, M. (2004). Facilitating effects of exercise on information processing. *Journal of Sports Sciences, 22*(5), 419-428.

Davranche, K., Audiffren, M., & Denjean, A. (2006). A distributional analysis of the effect of physical exercise on a choice reaction time task. *Journal of Sports Sciences, 24*(3), 323-329.

Delpy, D. T., & Cope, M. (1997). Quantification in tissue near-infrared spectroscopy. *Philosophical Transactions of the Royal Society B-Biological Sciences, 352*(1354), 649-659.

Dimpfel, W., Kler, A., Kriesl, E., Lenhfeld, R., & Keplinger-Dimpfel, I. K. (2006). Neurophysiological charachterisation of a functionally active drink containing extracts of ginkgo and ginseng by source density analysis of the human EEG. *Nutritional Neuroscience, 9*(5/6), 213-224.

Dommer, L., Jaeger, N., Scholkmann, F., Wolf, M., & Holper, L. (2012). Between-brain coherence during joint n-back task performance: A two-person functional near-infrared spectroscopy study. *Behavioural Brain Research, 234*(2), 212-222. doi: 10.1016/j.bbr.2012.06.024

Dove, A., Pollmann, S., Schubert, T., Wiggins, C. J., & Yves von Cramon, D. (2000). Prefrontal cortex activation in task switching: an event-related fMRI study. *Cognitive brain research, 9*(1), 103-109.

Drago, F., Floriddia, M. L., Cro, M., & Giuffrida, S. (2002). Pharmacokinetics and bioavailability of a Ginkgo biloba extract. *Journal of Ocular Pharmacology and Therapeutics, 18*(2), 197-202. doi: 10.1089/108076802317373941

Drummond, S. P. A., Brown, G. G., Stricker, J. L., Buxton, R. B., Wong, E. C., & Gillin, J. C. (1999). Sleep deprivation-induced reduction in cortical functional response to serial subtraction. *Neuroreport, 10*(18), 3745-3748. doi: 10.1097/00001756-199912160-00004

Dulloo, A. G., Geissler, C. A., Horton, T., Collins, A., & Miller, D. S. (1989). Normal Caffeine Consumption - Influence On Thermogenesis And Daily Energy-Expenditure In Lean And Postobese Human Volunteers. *American Journal of Clinical Nutrition, 49*(1), 44-50.

Ehlis, A.-C., Ringel, T., Plichta, M., Richter, M., Herrmann, M., & Fallgatter, A. (2009). Cortical correlates of auditory sensory gating: a simultaneous near-infrared spectroscopy event-related potential study. *Neuroscience, 159*(3), 1032-1043.

Einother, S. J. L., Martens, V. E. G., Rycroft, J. A., & De Bruin, E. A. (2010). L-Theanine and caffeine improve task switching but not intersensory attention or subjective alertness. *Appetite, 54*, 406-409.

Elsabagh, S., Hartley, D. E., Ali, O., Williamson, E. M., & File, S. E. (2005). Differential cognitive effects of Ginkgo biloba after acute and chronic treatment in healthy young volunteers. *Psychopharmacology, 179*(2), 437-446. doi: 10.1007/s00213-005-2206-6

Evans, S. M., & Griffiths, R. R. (1999). Caffeine withdrawal: A parametric analysis of caffeine dosing conditions. *Journal of Pharmacology and Experimental Therapeutics, 289*(1), 285-294.

Fallgatter, A. J., & Strik, W. K. (1998). Frontal brain activation during the Wisconsin Card Sorting Test assessed with two-channel near infrared spectroscopy. *European Archives of Psychiatry and Clinical Neuroscience, 248*, 245-249.

Fazli, S., Mehnert, J., Steinbrink, J., Curio, G., Villringer, A., Muller, K.-R., & Blankertz, B. (2012). Enhanced performance by a hybrid NIRS-EEG brain computer interface. *Neuroimage, 59*(1), 519-529. doi: http://dx.doi.org/10.1016/j.neuroimage.2011.07.084

Field, A. S., Laurienti, P. J., Yen, Y. F., Burdette, J. H., & Moody, D. M. (2003). Dietary caffeine consumption and withdrawal: Confounding variables in quantitative cerebral perfusion studies? *Radiology, 227*(1), 129-135. doi: 10.1148/radiol.2271012173

Fox, P. T., Raichle, M. E., Mintun, M. A., & Dence, C. (1988). Nonoxidative Glucose Consumption During Focal Physiologic Neural Activity. *Science, 241*, 462-464.

Francis, S. T., Head, K., Morris, P. G., & Macdonald, I. A. (2006). The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people. *Journal of cardiovascular pharmacology, 47*, S215-S220. doi: 10.1097/00005344-200606001-00018

Frayn, K. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology, 55*(2), 628-634.

Fredholm, B. B., Battig, K., Holmen, J., Nehlig, A., & Zvartau, E. E. (1999a). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Reviews, 51*(1), 83-133.

Fredholm, B. B., Battig, K., Holmen, J., Nehlig, A., & Zvartau, E. E. (1999b). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Reviews, 51*(1), 83-132.

Galduroz, J. C. F., Antunes, H. K., & Santos, R. F. (2007). Gender- and age-related variations in blood viscosity in normal volunteers: A study of the effects of extract of Allium sativum and Ginkgo biloba. *Phytomedicine, 14*(7-8), 447-451. doi: 10.1016/j.phymed.2007.06.002

Garcia-Peris, P., Lozano, M. A., Velasco, C., de La Cuerda, C., Iriondo, T., Breton, I., . . . Navarro, C. (2005). Prospective study of resting energy expenditure changes in head and neck cancer patients treated with chemoradiotherapy measured by indirect calorimetry. *Nutrition, 21*(11), 1107-1112.

Geng, J., Dong, J., Ni, H., Lee, M. S., Wu, T., Jiang, K., . . . Malouf, R. (2010). Ginseng for cognition. *Cochrane Database Syst Rev, 12*.

Gervain, J., Mehler, J., Werker, J. F., Nelson, C. A., Csibra, G., Lloyd-Fox, S., . . . Aslin, R. N. (2011). Near-infrared spectroscopy: a report from the McDonnell infant methodology consortium. *Developmental cognitive neuroscience, 1*(1), 22-46.

Gessner, B., Voelp, A., & Klasser, M. (1985). Study Of The Long-Term Action Of A Ginkgo Biloba Extract On Vigilance And Mental Performance As Determined By Means Of Quantitative Pharmaco-Eeg And Psychometric Measurements. *Arzneimittel-Forschung/Drug Research, 35-2*(9), 1459-1465.

Giesbrecht, T., Rycroft, J. A., Rowson, M. J., & De Bruin, E. A. (2010). The combination of L-theanine and caffeine improves cognitive performance and increases subjective alertness. *Nutritional Neuroscience, 13*(6), 283-290. doi: 10.1179/147683010x12611460764840

Glaister, M., Howatson, G., Abraham, C., Lockey, R., Goodwin, J., Foley, P., & Mcinnes, G. (2008). Caffeine supplementation and multiple sprint running performance. *Medicine+ Science in Sports+ Exercise, 40*(10), 1835.

Gold, P. E., Cahill, L., & Wenk, G. L. (2002). Ginkgo Biloba: A Cognitive Enhancer? *Psychological Science in the Public Interest, 3*(1), 2-11.

Gomez-Ramirez, M., Higgins, B. A., Rycroft, J. A., Owen, G. N., Mahoney, J., Shpaner, M., & Foxe, J. J. (2007). The deployment of intersensory selective attention: A high-density electrical mapping study of the effects of theanine. *Clinical Neuropharmacology, 30*(1), 25-38. doi: 10.1097/01.wnf.0000240940.13876.17

Gomez-Ramirez, M., Kelly, S. P., Montesi, J. L., & Foxe, J. J. (2009). The Effects of L-theanine on Alpha-Band Oscillatory Brain Activity During a Visuo-Spatial Attention Task. *Brain Topography, 22*(1), 44-51. doi: 10.1007/s10548-008-0068-z

Graham, T. E., Battram, D. S., Dela, F., El-Sohemy, A., & Thong, F. S. L. (2008). Does caffeine alter muscle carbohydrate and fat metabolism during exercise? *Applied Physiology Nutrition and Metabolism-Physiologie Appliquee Nutrition Et Metabolisme, 33*(6), 1311-1318. doi: 10.1139/h08-129

Greer, F., Friars, D., & Graham, T. E. (2000). Comparison of caffeine and theophylline ingestion: exercise metabolism and endurance. *Journal of Applied Physiology, 89*(5), 1837-1844.

Haller, S., Rodriguez, C., Moser, D., Toma, S., Hofmeister, J., Sinanaj, I., . . . Lovblad, K.-O. (2013). Acute caffeine administration impact on working memory-related brain activation and functional connectivity in the elderly: A BOLD and perfusion MRI study. *Neuroscience, 250*, 364-371.

Haskell, C., Dodd, F., Wightman, E., & Kennedy, D. (2013). Behavioural effects of compounds co-consumed in dietary forms of caffeinated plants. *Nutrition research reviews, 26*(01), 49-70.

Haskell, C., Kennedy, D., Milne, A., Wesnes, K., & Scholey, A. (2008a). Caffeine at levels found in decaffeinated beverages is behaviourally active. *Appetite, 50*(2-3), 559.

Haskell, C. F., Kennedy, D. O., Milne, A. L., Wesnes, K. A., & Scholey, A. B. (2008b). The effects of L-theanine caffeine and their combination on cognition and mood. *Biological psychology, 77*, 113-122.

Haskell, C. F., Kennedy, D. O., Wesnes, K. A., & Scholey, A. B. (2005). Cognitive and mood improvements of caffeine in habitual consumers and habitual non-consumers of caffeine. *Psychopharmacology, 179*(4), 813-825. doi: 10.1007/s00213-004-2104-3

Haskell, C. F., Robertson, B., Jones, E., Forster, J., Jones, R., Wilde, A., . . . Kennedy, D. O. (2010). Effects of a multi-vitamin/mineral supplement on cognitive function and fatigue during extended multi-tasking. *Hum Psychopharmacol, 25*(6), 448-461.

Hasse, C. G., Becka, M., Kuhlmann, J., & Wensing, G. (2005). Influences of caffeine, acetazolamide and cognitve stimulation on cerebral blood flow velocities. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 29*, 549-556.

Haugen, H. A., Chan, L.-N., & Li, F. (2007). Indirect calorimetry: a practical guide for clinicians. *Nutrition in Clinical Practice, 22*(4), 377-388.

Hayman, M. (1942). Two minute clinical test for measurement of intellectual impairment in psychiatric disorders. *Archives of Neurology and Psychiatry, 47*(3), 454-464.

Heese, T., Jenkinson, J., Love, C., Milam, R., Perkins, L., Adams, C., . . . Ceremuga, T. E. (2009). Anxiolytic effects of L-theanine--a component of green tea--when combined with midazolam, in the male Sprague-Dawley rat. *Aana J, 77*(6), 445-449.

Heilbronner, U., Hinrichs, H., Heinze, H.-J., & Zaehle, T. (2015). Caffeine differentially alters cortical hemodynamic activity during working memory: a near infrared spectroscopy study. *BMC Research Notes, 8*(1).

Herrmann, M., Ehlis, A.-C., Wagener, A., Jacob, C., & Fallgatter, A. (2005). Near-infrared optical topography to assess activation of the parietal cortex during a visuo-spatial task. *Neuropsychologia, 43*(12), 1713-1720.

Herrmann, M. J., Huter, T., Plichta, M. M., Ehlis, A. C., Alpers, G. W., Muhlberger, A., & Fallgatter, A. J. (2008). Enhancement of activity of the primary visual cortex during processing of emotional stimuli as measured with event-related functional near-

infrared spectroscopy and event-related potentials. *Human brain mapping, 29*(1), 28-35.

Hewlett, P., & Smith, A. (2006). Acute effects of caffeine in volunteers with different patterns of regular consumption. *Human Psychopharmacology-Clinical and Experimental, 21*(3), 167-180. doi: 10.1002/hup.752

Higashi, T., Sone, Y., Ogawa, K., Kitamura, Y. T., Saiki, K., Sagawa, S., . . . Seiyama, A. (2004). Changes in regional cerebral blood volume in frontal cortex during mental work with and without caffeine intake: functional monitoring near-infrared spectroscopy. *Journal of biomedical optics, 9*(4), 788-793.

Hodgson, A. B., Randell, R. K., & Jeukendrup, A. E. (2013). The Metabolic and Performance Effects of Caffeine Compared to Coffee during Endurance Exercise. *Plos One, 8*(4). doi: 10.1371/journal.pone.0059561

Hofferberth, B. (1994). The Efficacy Of Egb-761 In Patients With Senile Dementia Of The Alzheimer-Type, A Double-Blind, Placebo-Controlled Study On Different Levels Of Investigation. *Human Psychopharmacology-Clinical and Experimental, 9*(3), 215-222. doi: 10.1002/hup.470090308

Hogervorst, E., Bandelow, S., Schmitt, J., Jentjens, R., Oliveira, M., Allgrove, J., . . . Gleeson, M. (2008). Caffeine improves physical and cognitive performance during exhaustive exercise. *Medicine+ Science in Sports+ Exercise, 40*(10), 1841.

Hollands, M. A., Arch, J. R. S., & Cawthorne, M. A. (1981). A Simple Apparatus For Comparative Measurements Of Energy-Expenditure In Human-Subjects - The Thermic Effect Of Caffeine. *American Journal of Clinical Nutrition, 34*(10), 2291-2294.

Holper, L., Scholkmann, F., & Wolf, M. (2012). Between-brain connectivity during imitation measured by fNIRS. *Neuroimage, 63*(1), 212-222. doi: 10.1016/j.neuroimage.2012.06.028

Hoshi, Y. (2003). Functional near-infrared optical imaging: Utility and limitations in human brain mapping. *Psychophysiology, 40*(4), 511-520. doi: 10.1111/1469-8986.00053

Hoshi, Y. (2007). Functional near-infrared spectroscopy: current status and future prospects. *Journal of biomedical optics, 12*(6).

Huppert, T. J., Hoge, R. D., Diamond, S. G., Franceschini, M. A., & Boas, D. A. (2006). A temporal comparison of BOLD, ASL and NIRS hemodynamic responses to motor stimuli in adult humans. *Neuroimage, 29*, 368-382.

Ide, K., Horn, A., & Secher, N. H. (1999). Cerebral metabolic response to submaximal exercise. *Journal of Applied Physiology, 87*(5), 1604-1608.

Itil, T. M., Eralp, E., Ahmed, I., Kunitz, A., & Itil, K. Z. (1998). The pharmacological effects of Ginkgo biloba, a plant extract, on the brain of dementia patients in comparison with tacrine. *Psychopharmacology Bulletin, 34*(3), 391-397.

Itil, T. M., Itil, K. Z., Eralp, E., & Le Bars, P. (1996). Central nervous system effects of Ginkgo biloba, a plant extract. *American Journal of Therapeutics, 3*, 63-73.

Itoh, M., Hatazawa, J., Miyazawa, H., Matsui, H., Meguro, K., Yanai, K., . . . Matsuzawa, T. (1990). Stability of cerebral blood flow and oxygen metabolism during normal aging. *Gerontology, 36*(1), 43-48.

Izzetoglu, K., Bunce, S., Onaral, B., Pourrezaei, K., & Chance, B. (2004). Functional Optical Brain Imaging Using Near-Infrared During Cognitive Tasks. *International Journal of Human-Computer Interaction, 17*(2), 211-227.

Izzetoglu, K., Yurtsever, G., Bozkurt, A., & Bunce, S. (2003). Functional Brain Monitoring via NIR Based Optical Spectroscopy.

Izzetoglu, K., Yurtsever, G., Bozkurt, A., Yazici, B., Bunce, S., Pourrezaei, K., & Onaral, B. (2002). *NIR Spectroscopy Measurements of Cognitive load Elicited by GKT and Target Categorisation.* Paper presented at the Proceedings of the 36th Hawaii International Conference on System Sciences.

Izzetoglu, M., Devaraj, A., Bunce, S., & Onaral, B. (2005). Motion artifact cancellation in NIR spectroscopy using Wiener filtering. *IEEE Transactions On Biomedical Engineering, 52*(5), 934-938. doi: 10.1109/tbme.2005.845243

Jackson, P. A., Reay, J. L., Scholey, A. B., & Kennedy, D. O. (2012a). DHA-rich oil modulates the cerebral haemodynamic response to cognitive tasks in healthy

young adults: a near IR spectroscopy pilot study. *British Journal of Nutrition, 107*(8), 1093-1098. doi: 10.1017/s0007114511004041

Jackson, P. A., Reay, J. L., Scholey, A. B., & Kennedy, D. O. (2012b). Docosahexaenoic acid-rich fish oil modulates the cerebral hemodynamic response to cognitive tasks in healthy young adults. *Biological psychology, 89*(1), 183-190.

James, J. E. (2004). Critical review of dietary caffeine and blood pressure: A relationship that should be taken more seriously. *Psychosomatic Medicine, 66*(1), 63-71. doi: 10.1097/10.psy.0000107884.78247.f9

Jezova, D., Duncko, R., Lassanova, M., Kriska, M., & Moncek, F. (2002). Reduction of rise in blood pressure and cortisol release during stress by Ginko biloba extract (EGb 761) in healthy volunteers. *Journal of Physiology and Pharmacology, 53*(3), 337-348.

Johansson, B., Ahlberg, S., van der Ploeg, I., Brené, S., Lindefors, N., Persson, H., & Fredholm, B. B. (1993). Effect of long term caffeine treatment on A1 and A2 adenosine receptor binding and on mRNA levels in rat brain. *Naunyn-Schmiedeberg's archives of pharmacology, 347*(4), 407-414.

Joris, P. J., & Mensink, R. P. (2013). Beetroot juice improves in overweight and slightly obese men postprandial endothelial function after consumption of a mixed meal. *Atherosclerosis, 231*(1), 78-83. doi: 10.1016/j.atherosclerosis.2013.09.001

Juneja, L. R., Chu, D. C., Okubo, T., Nagato, Y., & Yokogoshi, H. (1999). L-theanine - a unique amino acid of green tea and its relaxation effect in humans. *Trends in Food Science & Technology, 10*(6-7), 199-204.

Jung, F., Mrowietz, C., Kiesewetter, H., & Wenzel, E. (1990). Effect Of Ginkgo-Biloba On Fluidity Of Blood And Peripheral Microcirculation In Volunteers. *Arzneimittel-Forschung/Drug Research, 40-1*(5), 589-593.

Kapil, V., Milsom, A. B., Okorie, M., Maleki-Toyserkani, S., Akram, F., Rehman, F., . . . Ahluwalia, A. (2010). Inorganic Nitrate Supplementation Lowers Blood Pressure in Humans Role for Nitrite-Derived NO. *Hypertension, 56*(2), 274-U174. doi: 10.1161/hypertensionaha.110.153536

Karelis, A. D., Smith, J. E. W., Passe, D. H., & Peronnet, F. (2010). Carbohydrate administration and exercise performance. *Sports Medicine, 40*(9), 747-763.

Keheyan, G., Dunn, L. A., & Hall, W. L. (2011). Acute Effects of Ginkgo Biloba Extract on Vascular Function and Blood Pressure. *Plant Foods for Human Nutrition, 66*(3), 209-211. doi: 10.1007/s11130-011-0234-4

Kehr, J., Yoshitake, S., Ijiri, S., Koch, E., Noldner, M., & Yoshitake, T. (2012). Ginkgo biloba leaf extract (EGb 761 (R)) and its specific acylated flavonol constituents increase dopamine and acetylcholine levels in the rat medial prefrontal cortex: possible implications for the cognitive enhancing properties of EGb 761 (R). *International Psychogeriatrics, 24*, S25-S34. doi: 10.1017/s1041610212000567

Kelly, J., Fulford, J., Vanhatalo, A., Blackwell, J. R., French, O., Bailey, S. J., . . . Jones, A. M. (2013). Effects of short-term dietary nitrate supplementation on blood pressure, O-2 uptake kinetics, and muscle and cognitive function in older adults. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 304*(2), R73-R83. doi: 10.1152/ajpregu.00406.2012

Kelly, S. P., Gomez-Ramirez, M., Montesi, J. L., & Foxe, J. J. (2008). L-theanine and caffeine in combination affect human cognition as evidenced by oscillatory alpha-band activity and attention task performance. *Journal of Nutrition, 138*(8), 1572-1577.

Kenjale, A. A., Ham, K. L., Stabler, T., Robbins, J. L., Johnson, J. L., VanBruggen, M., . . . Allen, J. D. (2011). Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease. *Journal of Applied Physiology, 110*(6), 1582-1591. doi: 10.1152/japplphysiol.00071.2011

Kennedy, D., & Haskell, C. (2011). Cerebral blood flow and behavioural effects of caffeine in habitual and non-habitual consumers of caffeine: A near infrared spectroscopy study. *Biological psychology, 86*, 298-306.

Kennedy, D., Reay, J., & Scholey, A. (2007). Effects of 8 weeks administration of Korean Panax ginseng extract on the mood and cognitive performance of healthy individuals. *Journal of Ginseng Research, 31*(1), 34-43.

Kennedy, D. O., Haskell, C. F., Mauri, P. L., & Scholey, A. B. (2007). Acute cognitive effects of standardised Ginkgo biloba extract complexed with phosphatidylserine. *Human Psychopharmacology-Clinical and Experimental, 22*(4), 199-210. doi: 10.1002/hup.837

Kennedy, D. O., Haskell, C. F., Wesnes, K. A., & Scholey, A. B. (2004). Improved cognitive performance in human volunteers following administration of guarana (Paullinia cupana) extract: comparison and interaction with Panax ginseng. *Pharmacology Biochemistry and Behavior, 79*(3), 401-411. doi: 10.1016/j.pbb.2004.07.014

Kennedy, D. O., Jackson, P. A., Haskell, C. F., & Scholey, A. B. (2007). Modulation of cognitive performance following single doses of 120 mg Ginkgo biloba extract administered to healthy young volunteers. *Human Psychopharmacology-Clinical and Experimental, 22*(8), 559-566. doi: 10.1002/hup.885

Kennedy, D. O., & Scholey, A. B. (2000). Glucose administration, heart rate and cognitive performance: effects of increasing mental effort. *Psychopharmacology, 149*(1), 63-71. doi: 10.1007/s002139900335

Kennedy, D. O., & Scholey, A. B. (2004). A glucose-caffeine 'energy drink' ameliorates subjective and performance deficits during prolonged cognitive demand. *Appetite, 42*(3), 331-333.

Kennedy, D. O., Scholey, A. B., Drewery, L., Marsh, V. R., Moore, B., & Ashton, H. (2003). Electroencephalograph effects of single doses of Ginkgo biloba and Panax ginseng, in healthy young volunteers. *Pharmacology, Biochemistry and Behavior, 75*, 701-709.

Kennedy, D. O., Scholey, A. B., & Wesnes, K. A. (2000). The dose-dependent cognitive effects of acute administration of Ginkgo biloba to healthy young volunteers. *Psychopharmacology, 151*(4), 416-423. doi: 10.1007/s002130000501

Kennedy, D. O., Scholey, A. B., & Wesnes, K. A. (2001a). Differential, dose dependent changes in cognitive performance following acute administration of a Ginkgo biloba/Panax ginseng combination to healthy young volunteers. *Nutritional Neuroscience, 4*(5), 399-412.

Kennedy, D. O., Scholey, A. B., & Wesnes, K. A. (2001b). Dose dependent changes in cognitive performance and mood following acute administration of Ginseng to healthy young volunteers. *Nutritional Neuroscience, 4*(4), 295-310.

Kennedy, D. O., Scholey, A. B., & Wesnes, K. A. (2002). Modulation of cognition and mood following administration of single doses of *Ginkgo biloba*, ginseng and a ginkgo/ginseng combination to healthy young adults. *Physiology & behavior, 75*, 739-751.

Kennedy, D. O., Stevenson, E. J., Jackson, P. A., Dunn, S., Wishart, K., Bieri, G., . . . Haskell-Ramsay, C., F. (2016). Multivitamins and minerals modulate whole-body energy metabolism and cerebral blood-flow during cognitive task performance: a double-blind, randomised, placebo-controlled trial. *Nutrition & Metabolism, 13*(11).

Kennedy, D. O., Veasey, R., Watson, A., Dodd, F., Jones, E., Maggini, S., & Haskell, C. F. (2010). Effects of high-dose B vitamin complex with vitamin C and minerals on subjective mood and performance in healthy males. *Psychopharmacology, 211*(1), 55-68. doi: 10.1007/s00213-010-1870-3

Kennedy, D. O., Wightman, E. L., Reay, J. L., Lietz, G., Okello, E. J., Wilde, A., & Haskell, C. F. (2010). Effects of resveratrol on cerebral blood flow variables and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation. *American Journal of Clinical Nutrition, 91*(6), 1590-1597. doi: 10.3945/ajcn.2009.28641

Keppel, G. (1991). *Design and analysis: a researcher's handbook* (3rd ed.). London: Prentice-Hall.

Kim, C. S., Park, J. B., Kim, K. J., Chang, S. J., Ryoo, S. W., & Jeon, B. H. (2002). Effect of Korea red ginseng on cerebral blood flow and superoxide production. *Acta Pharmacologica Sinica, 23*(12), 1152-1156.

Kimura, K., Ozeki, M., Juneja, L. R., & Ohira, H. (2007). L-Theanine reduces psychological and physiological stress responses. *Biological psychology, 74*(1), 39-45. doi: 10.1016/j.biopsycho.2006.06.006

Kimura, R., & Murata, T. (1971). Influence of alkylamides of glutamic acid and related compounds on central nervous system .1. Central depressant effect of theanine. *Chemical & Pharmaceutical Bulletin, 19*(6), 1257-&.

Kleijnen, J., & Knipschild, P. (1992). Ginkgo-Biloba for Cerebral Insufficiency. *British Journal of Clinical Pharmacology, 34*(4), 352-358.

Kleinschmidt, A., Obrig, H., Requardt, M., Merboldt, K.-D., Dirnagl, U., Villringer, A., & Frahm, J. (1996). Silmultaneous Recording of Cerebral Blood Oxygenation Chnages During Human Brain Activation by Magnetic Resonance Imaging and Near-Infrared Spectroscopy. *Journal of Cerebral Blood Flow and Metabolism, 16*, 817-826.

Klem, G. H., Lüders, H. O., Jasper, H., & Elger, C. (1999). The ten-twenty electrode system of the International Federation. *Electroencephalogr Clin Neurophysiol, 52*(3).

Knowles, R. G., & Moncada, S. (1994). Nitric-Oxide Synthases in Mammals. *Biochemical Journal, 298*, 249-258.

Knowles, R. G., Palacios, M., Palmer, R. M. J., & Moncada, S. (1989). Formation of Nitric-Oxide from L-Arginine in the Central Nervous-System - A Transduction Mechanism for Stimulation of the Soluble Guanylate-Cyclase. *Proceedings of the National Academy of Sciences of the United States of America, 86*(13), 5159-5162. doi: 10.1073/pnas.86.13.5159

Koch, E. (2005). Inhibition of platelet activating factor (PAF)-induced aggregation of human thrombocytes by ginkgolides: considerations on possible bleeding complications after oral intake of Ginkgo biloba extracts. *Phytomedicine, 12*(1-2), 10-16. doi: 10.1016/j.phymed.2004.02.002

Konagai, C., Watanabe, H., Abe, K., Tsuruoka, N., & Koga, Y. (2013). Effects of essence of chicken on cognitive brain function: a near-infrared spectroscopy study. *Bioscience, biotechnology, and biochemistry, 77*(1), 178-181.

Konagai, C., Yanagimoto, K., Hayamizu, K., Han, L., Tsuji, T., & Koga, Y. (2013). Effects of krill oil containing n-3 polyunsaturated fatty acids in phospholipid form on human brain function: a randomized controlled trial in healthy elderly volunteers. *Clinical interventions in aging, 8*, 1247.

Kotilahti, K., Nissila, I., Huotilainen, M., Makela, R., Gavrielides, N., Noponen, T., . . . Katila, T. (2005). Bilateral hemodynamic responses to auditory stimulation in newborn infants. *Neuroreport, 16*(12), 1373-1377. doi: 10.1097/01.wnr.0000175247.35837.15

Kringelbach, M. L., de Araujo, I. E. T., & Rolls, E. T. (2004). Taste-related activity in the human dorsolateral prefrontal cortex. *Neuroimage, 21*(2), 781-788. doi: 10.1016/j.neuroimage.2003.09.063

Kunkel, H. (1993). EEG Profile of 3 Different Extractions of Ginkgo-Biloba. *Neuropsychobiology, 27*(1), 40-45. doi: 10.1159/000118951

Lansley, K. E., Winyard, P. G., Bailey, S. J., Vanhatalo, A., Wilkerson, D. P., Blackwell, J. R., . . . Jones, A. M. (2011). Acute dietary nitrate supplementation improves cycling time trial performance. *Med Sci Sports Exerc, 43*(6), 1125-1131.

Lansley, K. E., Winyard, P. G., Fulford, J., Vanhatalo, A., Bailey, S. J., Blackwell, J. R., . . . Jones, A. M. (2011). Dietary nitrate supplementation reduces the O-2 cost of walking and running: a placebo-controlled study. *Journal of Applied Physiology, 110*(3), 591-600. doi: 10.1152/japplphysiol.01070.2010

Larsson, A., Skoog, I., Aevarsson, O., Arlig, A., Jacobsson, L., Larsson, L., . . . Wikkelso, C. (2001). Regional cerebral blood flow in normal individuals aged 40, 75 and 88 years studied by 99Tcm-d, l-HMPAO SPET. *Nuclear medicine communications, 22*(7), 741-746.

Laurienti, P. J., Field, A. S., Burdette, J. H., Maldjian, J. A., Yen, Y.-F., & Moody, D. M. (2002). Dietary Caffeine Consumption Modulates fMRI Measures. *Neuroimage, 17*(2), 751-757.

Laurienti, P. J., Field, A. S., Burdette, J. H., Maldjian, J. A., Yen, Y. F., & Moody, D. M. (2003). Relationship between caffeine-induced changes in resting cerebral perfusion and blood oxygenation level-dependent signal. *American Journal of Neuroradiology, 24*(8), 1607-1611.

Lawrence, N. S., Ross, T. J., & Stein, E. A. (2002). Cognitive mechanisms of nicotine on visual attention. *Neuron, 36*(3), 539-548. doi: 10.1016/s0896-6273(02)01004-8

LeBars, P. L., Katz, M. M., Berman, N., Itil, T. M., Freedman, A. M., & Schatzberg, A. F. (1997). A placebo-controlled, double-blind, randomized trial of an extract of Ginkgo biloba for dementia. *Jama-Journal of the American Medical Association, 278*(16), 1327-1332. doi: 10.1001/jama.278.16.1327

Lee, S.-T., Chu, K., Sim, J.-Y., Heo, J.-H., & Kim, M. (2008). Panax ginseng enhances cognitive performance in Alzheimer disease. *Alzheimer Disease & Associated Disorders, 22*(3), 222-226.

Leenders, K. L., Perani, D., Lammertsma, A. A., Heather, J. D., Buckingham, P., Healy, M. J. R., . . . Jones, T. (1990). Cerebral Blood-Flow, Blood-Volume and Oxygen Utilization - Normal Values and Effect of Age. *Brain, 113*, 27-47. doi: 10.1093/brain/113.1.27

Lefferts, W. K., Hughes, W. E., White, C. N., Brutsaert, T. D., & Heffernan, K. S. (2015). Effect of acute nitrate supplementation on neurovascular coupling and cognitive performance in hypoxia. *Applied Physiology, Nutrition, and Metabolism, 41*(999), 1-9.

Lieberman, H. R. (2001). The effects of ginseng, ephedrine, and caffeine on cognitive performance, mood and energy. *Nutrition Reviews, 59*(4), 91-102.

Lieberman, H. R., Falco, C. M., & Slade, S. S. (2002). Carbohydrate administration during a day of sustained aerobic activity improves vigilance, as assessed by a novel ambulatory monitoring device, and mood. *The American journal of clinical nutrition, 76*(1), 120-127.

Lieberman, H. R., Kanarek, R., B, & Prasad, C. (Eds.). (2005). *Nutritional Neuroscience*. Boca Raton, Florida: CRC Press.

Lieberman, H. R., Wurtman, R. J., Emde, G. G., Roberts, C., & Coviella, I. L. G. (1987). The Effects of Low-doses of Caffeine on Human-Performance and Mood. *Psychopharmacology, 92*(3), 308-312. doi: 10.1007/bf00210835

Lloyd-Fox, S., Blasi, A., & Elwell, C. E. (2009). Illuminating the developing brain: The past, present and future of functional near infrared spectroscopy. *Neuroscience and Biobehavioural Reviews, In press*.

Luck, S. J. (2014). *An introduction to the event-related potential technique*: MIT press.

Lundberg, J. O., & Govoni, M. (2004). Inorganic nitrate is a possible source for systemic generation of nitric oxide. *Free Radical Biology and Medicine, 37*(3), 395-400. doi: 10.1016/j.freeradbiomed.2004.04.027

Lundberg, J. O., & Weitzberg, E. (2010). NO-synthase independent NO generation in mammals. *Biochemical and Biophysical Research Communications, 396*(1), 39-45. doi: 10.1016/j.bbrc.2010.02.136

Lundberg, J. O., Weitzberg, E., & Gladwin, M. T. (2008). The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nature Reviews Drug Discovery, 7*(2), 156-167. doi: 10.1038/nrd2466

Mahadevan, S., & Park, Y. (2008). Multifaceted therapeutic benefits of Ginkgo biloba L.: Chemistry, efficacy, safety, and uses. *Journal of Food Science, 73*(1), R14-R19. doi: 10.1111/j.1750-3841.2007.00597.x

Maitra, I., Marcocci, L., Droylefaix, M. T., & Packer, L. (1995). Peroxyl Radical Scavenging Activity of Ginkgo-Biloba Extract EGB 761. *Biochemical Pharmacology, 49*(11), 1649-1655. doi: 10.1016/0006-2952(95)00089-i

Marchal, G., Rioux, P., Petit-Taboue, M.-C., Sette, G., Travere, J.-M., Le Poec, C., . . . Baron, J.-C. (1992). Regional cerebral oxygen consumption, blood flow, and blood volume in healthy human aging. *Archives of neurology, 49*(10), 1013-1020.

Martin, A. J., Friston, K. J., Colebatch, J. G., & Frackowiak, R. S. J. (1991). Decreases in Regional Cerebral Blood-Flow with Normal Ageing. *Journal of Cerebral Blood Flow and Metabolism, 11*(4), 684-689.

Mashayekh, A., Pham, D. L., Yousem, D. M., Dizon, M., Barker, P. B., & Lin, D. D. M. (2011). Effects of Ginkgo biloba on cerebral blood flow assessed by quantitative MR perfusion imaging: a pilot study. *Neuroradiology, 53*(3), 185-191. doi: 10.1007/s00234-010-0790-6

Masschelein, E., Van Thienen, R., Wang, X., Van Schepdael, A., Thomis, M., & Hespel, P. (2012). Dietary nitrate improves muscle but not cerebral oxygenation status during exercise in hypoxia. *Journal of Applied Physiology, 113*(5), 736-745. doi: 10.1152/japplphysiol.01253.2011

Mathew, R., & Wilson, W. (1985). Caffeine induced changes in cerebral circulation. *Stroke, 16*(5), 814-817.

Mathew, R. J., & Wilson, W. H. (1991). Substance Abuse and Cerebral Blood Flow. *The American Journal of Psychiatry, 148*(3), 292-305.

McMorris, T., & Graydon, J. (1997). The effect of exercise on cognitive performance in soccer-specific tests. *Journal of Sports Sciences, 15*(5), 459-468.

Medvedev, A. V., Kainerstorfer, J. M., Borisov, S. V., & VanMeter, J. (2011). Functional connectivity in the prefrontal cortex measured by near-infrared spectroscopy during ultrarapid object recognition. *Journal of biomedical optics, 16*(1), 016008-016008-016010.

Mehler, B., Reimer, B., & Coughlin, J. F. (2012). Sensitivity of Physiological Measures for Detecting Systematic Variations in Cognitive Demand From a Working Memory Task: An On-Road Study Across Three Age Groups. *Human Factors, 54*(3), 396-412. doi: 10.1177/0018720812442086

Melamed, E., Lavy, S., Bentin, S., Cooper, G., & Rinot, Y. (1980). Reduction in regional cerebral blood flow during normal aging in man. *Stroke, 11*(1), 31-35.

Milham, M. P., Erickson, K. I., Banich, M. T., Kramer, A. F., Webb, A., Wszalek, T., & Cohen, N. J. (2002). Attentional control in the aging brain: insights from an fMRI study of the stroop task. *Brain and cognition, 49*(3), 277-296.

Miller, M. D., Daniels, L. A., Bannerman, E., & Crotty, M. (2005). Resting energy expenditure measured longitudinally following hip fracture compared to predictive equations: is an injury adjustment required? *British Journal of Nutrition, 94*(6), 976-982.

Mix, J. A., & Crews, W. D. (2000). An examination of the efficacy of Ginkgo biloba extract EGb 761 on the neuropsychologic functioning of cognitively intact older adults. *Journal of Alternative and Complementary Medicine, 6*(3), 219-229. doi: 10.1089/acm.2000.6.219

Mix, J. A., & Crews, W. D. (2002). A double-blind, placebo-controlled, randomized trial of Ginkgo biloba extract EGb 761 (R) in a sample of cognitively intact older adults: neuropsychological findings. *Human Psychopharmacology-Clinical and Experimental, 17*(6), 267-277. doi: 10.1002/hup.412

Miyai, I., Tanabe, H. C., Sase, I., Eda, H., Oda, I., Konishi, I., . . . Kubota, K. (2001). Cortical mapping of gait in humans: a near-infrared spectroscopic topography study. *Neuroimage, 14*(5), 1186-1192.

Moerman, A., & De Hert, S. (2015). Cerebral oximetry: the standard monitor of the future? . *Current Opinion in Anaesthesiology*.

Molavi, B., & Dumont, G. A. (2012). Wavelet-based motion artifact removal for functional near-infrared spectroscopy. *Physiological measurement, 33*(2), 259.

Moncada, S., & Higgs, A. (1993). Mechanisms of Disease - The L-Arginine Nitric-Oxide Pathway. *New England Journal of Medicine, 329*(27), 2002-2012.

Moss, M. C., & Scholey, A. B. (1996). Oxygen administration enhances memory formation in healthy young adults. *Psychopharmacology, 124*(3), 255-260.

Moss, M. C., Scholey, A. B., & Wesnes, K. (1998). Oxygen administration selectively enhances cognitive performance in healthy young adults: a placebo controlled double blind crossover study. *Psychopharmacology, 138*(1), 27-33. doi: 10.1007/s002130050641

Mulderink, T. A., Gitelman, D. R., Mesulam, M. M., & Parrish, T. B. (2002). On the use of caffeine as a contrast booster for BOLD fMRI studies. *Neuroimage, 15*(1), 37-44. doi: 10.1006/nimg.2001.0973

Nakano, T., Watanabe, H., Homae, F., & Taga, G. (2009). Prefrontal Cortical Involvement in Young Infants' Analysis of Novelty. *Cerebral Cortex, 19*(2), 455-463. doi: 10.1093/cercor/bhn096

Nathan, P. J., Lu, K., Gray, M., & Oliver, C. (2006). The neuropharmacology of L-theanine(N-ethyl-L-glutamine): A possible neuroprotective and cognitive enhancing agent. *Journal of Herbal Pharmacotherapy, 6*(2), 21-30.

Neri, M., Andermarcher, E., Pradelli, J. M., & Salvioli, G. (1995). Influence of a double blind pharmacological trial on two domains of well-being in subjects with age associated memory impairment. *Archives of gerontology and geriatrics, 21*(3), 241-252.

NHS. (2011). *Supplements Who needs them?*

Niioka, T., & Sasaki, M. (2003). Individual cerebral hemodynamic response to caffeine was related to performance on a newly developed Stroop color-word task. *Optical Review, 10*(6), 607-608.

Nobre, A. C., Rao, A., & Owen, G. N. (2008). L-theanine, a natural constituent in tea, and its effect on mental state. *Asia Pacific Journal of Clinical Nutrition, 17*, 167-168.

Noordzij, M., Uiterwaal, C. S., Arends, L. R., Kok, F. J., Grobbee, D. E., & Geleijnse, J. M. (2005). Blood pressure response to chronic intake of coffee and caffeine: a meta-analysis of randomized controlled trials. *Journal of hypertension, 23*(5), 921-928.

Nurminen, M.-L., Niittynen, L., Korpela, R., & Vapaatalo, H. (1999). Coffee, caffeine and blood pressure: a critical review. *European journal of clinical nutrition, 53*(11), 831-839.

Obrig, H., Hirth, C., Junge-Hulsing, J., Doge, C., Wolf, T., Dirnagl, U., & Villringer, A. (1996). Cerebral oxygenation changes in response to motor stimulation. *Journal of Applied Physiology, 81*(3), 1174-1183.

Obrig, H., Israel, H., Kohl-Bareis, M., Uludag, K., Wenzel, R., Muller, B., . . . Villringer, A. (2002). Habituation of the visually evoked potential and its vascular response: Implications for neurovascular coupling in the healthy adult. *Neuroimage, 17*(1), 1-18. doi: 10.1006/nimg.2002.1177

Obrig, H., & Villringer, A. (2003). Beyond Visible - Imaging the Human Brain With Light. *Journal of Cerebral Blood Flow and Metabolism, 23*(1), 1-18.

Obrig, H., Wenzel, R., Kohl, M., Horst, S., Wobst, P., Steinbrink, J., . . . Villringer, A. (2000). Near-infrared spectroscopy: does it function in functional activation studies of the adult brain? *International Journal of Psychophysiology, 35*(2-3), 125-142.

Ogawa, S., Menon, R. S., Tank, D. W., Kim, S. G., Merkle, H., Ellermann, J. M., & Ugurbil, K. (1993). Functional brain mapping by blood oxygenation level-dependent contrast magnetic resonance imaging. A comparison of signal characteristics with a biophysical model. *Biophysical journal, 64*(3), 803-812.

Owen, A. M., McMillan, K. M., Laird, A. R., & Bullmore, E. (2005). N-back working memory paradigm: A meta-analysis of normative functional neuroimaging. *Human brain mapping, 25*(1), 46-59. doi: 10.1002/hbm.20131

Owen, G. N., Parnell, H., De Bruin, E. A., & Rycroft, J. A. (2008). The combined effects of L-theanine and caffeine on cognitive performance and mood. *Nutritional Neuroscience, 11*(4), 193-198. doi: 10.1179/147683008x301513

Palmer, R. M. J., Ashton, D. S., & Moncada, S. (1988). Vascular Endothelial-Cells Synthesize Nitric-Oxide From L-Arginine. *Nature, 333*(6174), 664-666. doi: 10.1038/333664a0

Pantano, P., Baron, J. C., Lebrungrandie, P., Duquesnoy, N., Bousser, M. G., & Comar, D. (1984). Regional Cerebral Blood-Flow And Oxygen-Consumption In Human Aging. *Stroke, 15*(4), 635-641.

Park, J. D., Rhee, D. K., & Lee, Y. H. (2005). Biological activities and chemistry of saponins from Panax ginseng C. A. Meyer. *Phytochemistry Reviews, 4*(2-3), 159-175. doi: 10.1007/s11101-005-2835-8

Pasman, W. J., Vanbaak, M. A., Jeukendrup, A. E., & Dehaan, A. (1995). The Effect Of Different Dosages Of Caffeine On Endurance Performance Time. *International Journal of Sports Medicine, 16*(4), 225-230. doi: 10.1055/s-2007-972996

Patwardhan, R. V., Desmond, P. V., Johnson, R. F., & Schenker, S. (1980). Impaired Elimination Of Caffeine By Oral-Contraceptive Steroids. *Journal of Laboratory and Clinical Medicine, 95*(4), 603-608.

Perthen, J. E., Lansing, A. E., Liau, J., Liu, T. T., & Buxton, R. B. (2008). Caffeine-induced uncoupling of cerebral blood flow and oxygen metabolism: A calibrated BOLD fMRI study. *Neuroimage, 40*(1), 237-247. doi: 10.1016/j.neuroimage.2007.10.049

Piknova, B., Kocharyan, A., Schechter, A. N., & Silva, A. C. (2011). The role of nitrite in neurovascular coupling. *Brain Research, 1407*, 62-68. doi: 10.1016/j.brainres.2011.06.045

Presley, T. D., Morgan, A. R., Bechtold, E., Clodfelter, W., Dove, R. W., Jennings, J. M., . . . Miller, G. D. (2011). Acute effect of a high nitrate diet on brain perfusion in older adults. *Nitric Oxide-Biology and Chemistry, 24*(1), 34-42. doi: 10.1016/j.niox.2010.10.002

Quinlan, P. T., Lane, J., Moore, K. L., Aspen, J., Rycroft, J. A., & O'Brien, D. C. (2000). The acute physiological and mood effects of tea and coffee: The role of caffeine level. *Pharmacology Biochemistry and Behavior, 66*(1), 19-28.

Rack-Gomer, A. L., Liau, J., & Liu, T. T. (2009). Caffeine reduces resting-state BOLD functional connectivity in the motor cortex. *Neuroimage, 46*(1), 56-63. doi: :10.1016/j.neuroimage.2009.02.001

Radomski, M. W., Palmer, R. M. J., & Moncada, S. (1990). An L-Arginine Nitric-Oxide Pathway Present In Human Platelets Regulates Aggregation. *Proceedings of the National Academy of Sciences of the United States of America, 87*(13), 5193-5197. doi: 10.1073/pnas.87.13.5193

Ramassamy, C., Christen, Y., Clostre, F., & Costentin, J. (1992). The Ginkgo-Biloba Extract, Egb761, Increases Synaptosomal Uptake Of 5-Hydroxytryptamine - Invitro And Exvivo Studies. *Journal of Pharmacy and Pharmacology, 44*(11), 943-945.

Rattray, B., Egle-Marshall, L., Northey, J., Hone, S., Smee, D., & Brassard, P. (2015). Dietary Nitrate improves Cerebral Perfusion, in Young Adults during Exercise: Relationship to Cognitive Performance. *The FASEB Journal, 29*(1 Supplement), 989.982.

Reaburn, P., Reed, R., Dascombe, B. J., Jones, A., & Weyers, J. D. (2011). *Practical Skills in Sport and Exercise Science*: Prentice Hall.

Reay, J. L., Kennedy, D. O., & Scholey, A. B. (2005). Single doses of Panax ginseng (G115) reduce blood glucose levels and improve cognitive performance during sustained mental activity. *Journal of Psychopharmacology, 19*(4), 357-365. doi: 10.1177/0269881105053286

Reay, J. L., Kennedy, D. O., & Scholey, A. B. (2006). Effects of Panax ginseng, consumed with and without glucose, on blood glucose levels and cognitive performance during sustained 'mentally demanding' tasks. *Journal of Psychopharmacology, 20*(6), 771-781. doi: 10.1177/0269881106061516

Reay, J. L., Scholey, A. B., & Kennedy, D. O. (2010). Panax ginseng (G115) improves aspects of working memory performance and subjective ratings of calmness in healthy young adults. *Human Psychopharmacology: Clinical and Experimental, 25*(6), 462-471.

Reeves, M. M., Battistutta, D., Capra, S., Bauer, J., & Davies, P. S. (2006). Resting energy expenditure in patients with solid tumors undergoing anticancer therapy. *Nutrition, 22*(6), 609-615.

Riby, L. M., Sunram-Lea, S. I., Graham, C., Foster, J. K., Cooper, T., Moodie, C., & Gunn, V. P. (2008). P3b versusP3a: an event-related potential investigation of the glucose facilitation effect. *Journal of Psychopharmacology, 22*(5), 486-492.

Richardson, N. J., Rogers, P. J., Elliman, N. A., & Odell, R. J. (1995). Mood And Performance Effects Of Caffeine In Relation To Acute And Chronic Caffeine Deprivation. *Pharmacology Biochemistry and Behavior, 52*(2), 313-320. doi: 10.1016/0091-3057(95)00029-v

Rifkind, J. M., Nagababu, E., Barbiro-Michaely, E., Ramasamy, S., Pluta, R. M., & Mayevsky, A. (2007). Nitrite infusion increases cerebral blood flow and decreases mean arterial blood pressure in rats: a role for red cell NO. *Nitric oxide : biology and chemistry / official journal of the Nitric Oxide Society, 16*(4), 448-456. doi: 10.1016/j.niox.2007.04.002

Rissman, J., Gazzaley, A., & D'Esposito, M. (2004). Measuring functional connectivity during distinct stages of a cognitive task. *Neuroimage, 23*(2), 752-763.

Robertson, F. C., Douglas, T. S., & Meintjes, E. M. (2010). Motion artifact removal for functional near infrared spectroscopy: a comparison of methods. *Biomedical Engineering, IEEE Transactions on, 57*(6), 1377-1387.

Roche-Labarbe, N., Wallois, F., Ponchel, E., Kongolo, G., & Grebe, R. (2007). Coupled oxygenation oscillation measured by NIRS and intermittent cerebral activation on EEG in premature infants. *Neuroimage, 36*(3), 718-727.

Roche-Labarbe, N., Zaaimi, B., Berquin, P., Nehlig, A., Grebe, R., & Wallois, F. (2008). NIRS-measured oxy- and deoxyhemoglobin changes associated with EEG spike-and-wave discharges in children. *Epilepsia, 49*(11), 1871-1880. doi: 10.1111/j.1528-1167.2008.01711.x

Rogers, P. J. (2007). Caffeine, mood and mental performance in everyday life. *Nutrition Bulletin, 32*(Suppl 1), 84-89.

Rogers, P. J., Martin, J., Smith, C., Heatherley, S. V., & Smit, H. J. (2003). Absence of reinforcing, mood and psychomotor performance effects of caffeine in habitual non-consumers of caffeine. *Psychopharmacology, 167*(1), 54-62. doi: 10.1007/s00213-002-1360-3

Rogers, P. J., Smith, J. E., Heatherley, S. V., & Pleydell-Pearce, C. W. (2008). Time for tea: mood, blood pressure and cognitive performance effects of caffeine and theanine administered alone and together. *Psychopharmacology, 195*, 569-577. doi: 10.1007/s00213-007-0938-1

Rooks, C. R., Thom, N. J., McCully, K. K., & Dishman, R. K. (2010). Effects of incremental exercise on cerebral oxygenation measured by near-infrared spectroscopy: A systematic review. *Progress in Neurobiology, 92*(2), 134-150. doi: 10.1016/j.pneurobio.2010.06.002

Saffle, J. R., Medina, E., Raymond, J., Westenskow, D., Kravitz, M., & Warden, G. D. (1985). Use of indirect calorimetry in the nutritional management of burned patients. *Journal of Trauma-Injury, Infection, and Critical Care, 25*(1), 32-39.

Sakatani, K., Chen, S., Lichty, W., Zuo, H., & Wang, Y.-p. (1999). Cerebral blood oxygenation changes induced by auditory stimulation in newborn infants measured by near infrared spectroscopy. *Early Human Development, 55*(3), 229-236.

Sakatani, K., Tanida, M., Hirao, N., & Takemura, N. (2014). Ginkobiloba extract improves working memory performance in middle-aged women: role of asymmetry of prefrontal cortex activity during a working memory task *Oxygen Transport to Tissue XXXVI* (pp. 295-301): Springer.

Salinsky, M., Kanter, R., & Dasheiff, R. M. (1987). Effectiveness of multiple EEGs in supporting the diagnosis of epilepsy: an operational curve. *Epilepsia, 28*(4), 331-334.

Salthouse, T. A. (1991). Mediation of adult age differences in cognition by reductions in working memory and speed of processing. *Psychological Science, 2*(3), 179-183.

Salthouse, T. A. (2009). When does age-related cognitive decline begin? *Neurobiology of aging, 30*(4), 507-514.

Salthouse, T. A., & Babcock, R. L. (1991). Decomposing adult age differences in working memory. *Developmental psychology, 27*(5), 763.

Santamaria, P., Elia, A., Serio, F., & Todaro, E. (1999). A survey of nitrate and oxalate content in fresh vegetables. *Journal of the Science of Food and Agriculture, 79*(13), 1882-1888. doi: 10.1002/(sici)1097-0010(199910)79:13<1882::aid-jsfa450>3.3.co;2-4

Santos, R. F., Galduroz, J. C. F., Barbieri, A., Castiglioni, M. L. V., Ytaya, L. Y., & Bueno, O. F. A. (2003). Cognitive performance, SPECT, and blood viscosity in elderly non-demented people using Ginkgo biloba. *Pharmacopsychiatry, 36*(4), 127-133.

Sato, H., Yahata, N., Funane, T., Takizawa, R., Katura, T., Atsumori, H., . . . Kasai, K. (2013). A NIRS-fMRI investigation of prefrontal cortex activity during a working memory task. *Neuroimage, 83*, 158-173. doi: 10.1016/j.neuroimage.2013.06.043

Sato, Y., Mori, K., Koizumi, T., Minagawa-Kawai, Y., Tanaka, A., Ozawa, E., . . . Mazuka, R. (2011). Functional lateralization of speech processing in adults and children who stutter. *Frontiers in psychology, 2*, 70.

Schmitt, J. A. J., Benton, D., & Kallus, K. W. (2005). General methodological considerations for the assessment of nutritional influences on human cognitive

functions. *European Journal of Nutrition, 44*(8), 459-464. doi: 10.1007/s00394-005-0585-4

Scholey, A. (2001). Fuel for thought. *Psychologist, 14*(4), 196-201.

Scholey, A. B., French, S. J., Morris, P. J., Kennedy, D. O., Milne, A. L., & Haskell, C. F. (2009). Consumption of cocoa flavanols results in acute improvements in mood and cognitive performance during sustained mental effort. *Journal of Psychopharmacology, 24*(10), 1505-1514. doi: 10.1177/0269881109106923

Scholey, A. B., Harper, S., & Kennedy, D. O. (2001). Cognitive demand and blood glucose. *Physiology & behavior, 73*(4), 585-592. doi: 10.1016/s0031-9384(01)00476-0

Scholey, A. B., Moss, M. C., Neave, N., & Wesnes, K. (1999). Cognitive performance, hyperoxia, and heart rate following oxygen administration in healthy young adults. *Physiology & behavior, 67*(5), 783-789. doi: 10.1016/s0031-9384(99)00183-3

Schroeter, M. L., Kupka, T., Mildner, T., Uludag, K., & Von Cramon, D. Y. (2006). Investigating the post-stimulus undershoot of the BOLD signal - a simultaneous fMRI and fNIRS study. *Neuroimage, 30*(2), 349-358.

Schroeter, M. L., Zysset, S., Kupka, T., Kruggel, F., & Yves von Cramon, D. (2002). Near-Infrared Spectroscopy Can Detect Brain Activity During a Color-Word Matching Stroop Task in an Event-Related Design. *Human brain mapping, 17*, 61-71.

Seematter, G., Guenat, E., Schneiter, P., Cayeux, C., Jéquier, E., & Tappy, L. (2000). Effects of mental stress on insulin-mediated glucose metabolism and energy expenditure in lean and obese women. *American Journal of Physiology-Endocrinology and Metabolism, 279*(4), E799-E805.

Semlitsch, H. V., Anderer, P., Saletu, B., Binder, G. A., & Decker, K. A. (1995). Cognitive Psychophysiology In Nootropic Drug Research - Effects Of Ginkgo-Biloba On Event-Related Potentials (P300) In Age-Associated Memory Impairment. *Pharmacopsychiatry, 28*(4), 134-142. doi: 10.1055/s-2007-979605

Sengupta, S., Toh, S. A., Sellers, L. A., Skepper, J. N., Koolwijk, P., Leung, H. W., . . . Fan, T. P. D. (2004). Modulating angiogenesis - The yin and the yang in ginseng. *Circulation, 110*(10), 1219-1225. doi: 10.1161/01.cir.0000140676.88412.cf

Shibuya-Tayoshi, S., Sumitani, S., Kikuchi, K., Tanaka, T., Tayoshi, S. y., Ueno, S.-I., & Ohmori, T. (2007). Activation of the prefrontal cortex during the Trail-Making Test detected with multichannel near-infrared spectroscopy. *Psychiatry and Clinical Neurosciences, 61*, 616-621.

Silberstein, R. B., Pipingas, A., Song, J., Camfield, D. A., Nathan, P. J., & Stough, C. (2011). Examining Brain-Cognition Effects of Ginkgo Biloba Extract: Brain Activation in the Left Temporal and Left Prefrontal Cortex in an Object Working Memory Task. *Evidence-Based Complementary and Alternative Medicine*. doi: 10.1155/2011/164139

Simmonds, M. J., Minahan, C. L., & Sabapathy, S. (2010). Caffeine improves supramaximal cycling but not the rate of anaerobic energy release. *European journal of applied physiology, 109*(2), 287-295. doi: 10.1007/s00421-009-1351-8

Small, D. M., Gregory, M. D., Mak, Y. E., Gitelman, D., Mesulam, M. M., & Parrish, T. (2003). Dissociation of neural representation of intensity and affective valuation in human gustation. *Neuron, 39*(4), 701-711. doi: 10.1016/s0896-6273(03)00467-7

Smit, H. J., & Rogers, P. J. (2000). Effects of low doses of caffeine on cognitive performance, mood and thirst in low and higher caffeine consumers. *Psychopharmacology, 152*(2), 167-173. doi: 10.1007/s002130000506

Smith, A. P., Christopher, G., & Sutherland, D. (2013). Acute effects of caffeine on attention: a comparison of non-consumers and withdrawn consumers. *Journal of Psychopharmacology, 27*(1), 77-83. doi: 10.1177/0269881112460112

Smith, M. A., Riby, L. M., Sunram-Lea, S. I., Van Eekelen, J. A. M., & Foster, J. K. (2009). Glucose modulates event-related potential components of recollection and familiarity in healthy adolescents. *Psychopharmacology, 205*(1), 11-20.

Smith, P. F., Maclennan, K., & Darlington, C. L. (1996). The neuroprotective properties of the Ginkgo biloba leaf: A review of the possible relationship to platelet-activating factor (PAF). *Journal of ethnopharmacology, 50*(3), 131-139. doi: 10.1016/0378-8741(96)01379-7

Smith, S. J. M. (2005). EEG in the diagnosis, classification, and management of patients with epilepsy. *Journal of Neurology, Neurosurgery & Psychiatry, 76*(suppl 2), ii2-ii7.

Smits, M., Peeters, R. R., van Hecke, P., & Sunaert, S. (2007). A 3 T event-related functional magnetic resonance imaging (fMRI) study of primary and secondary gustatory cortex localization using natural tastants. *Neuroradiology, 49*(1), 61-71. doi: 10.1007/s00234-006-0160-6

Sorensen, H., & Sonne, J. (1996). A double-masked study of the effects of ginseng on cognitive functions. *Current Therapeutic Research-Clinical and Experimental, 57*(12), 959-968. doi: 10.1016/s0011-393x(96)80114-7

Spriet, L. L., Maclean, D. A., Dyck, D. J., Hultman, E., Cederblad, G., & Graham, T. E. (1992). Caffeine Ingestion And Muscle Metabolism During Prolonged Exercise In Humans. *American Journal of Physiology, 262*(6), E891-E898.

Stough, C., Clarke, J., Lloyd, J., & Nathan, P. J. (2001). Neuropsychological changes after 30-day Ginkgo biloba administration in healthy participants. *International Journal of Neuropsychopharmacology, 4*(2), 131-134.

Strangman, G., Culver, J. P., Thompson, J. H., & Boas, D. A. (2002). A quantitative comparison of simultaneous BOLD fMRI and NIRS recordings during functional brain activation. *Neuroimage, 17*(2), 719-731. doi: 10.1006/nimg.2002.1227

Stromgaard, K., Saito, D. R., Shindou, H., Ishii, S., Shimizu, T., & Nakanishi, K. (2002). Ginkgolide derivatives for photolabeling studies: Preparation and pharmacological evaluation. *Journal of Medicinal Chemistry, 45*(18), 4038-4046. doi: Unsp jm020147w10.1021/jm020147w

Subudhi, A. W., Dimmen, A. C., & Roach, R. C. (2007). Effects of acute hypoxia on cerebral and muscle oxygenation during incremental exercise. *Journal of Applied Physiology, 103*(1), 177-183.

Subudhi, A. W., Miramon, B. R., Granger, M. E., & Roach, R. C. (2009). Frontal and motor cortex oxygenation during maximal exercise in normoxia and hypoxia. *Journal of Applied Physiology, 106*(4), 1153-1158.

Sunram-Lea, S., Birchall, R., Wesnes, K., & Petrini, O. (2005). The effect of acute administration of 400 mg of Panax ginseng on cognitive performance and mood in healthy young volunteers. *Current Topics in Nutraceutical Research, 3*(1), 65-74.

Suzuki, M., Miyai, I., Ono, T., Oda, I., Konishi, I., Kochiyama, T., & Kubota, K. (2004). Prefrontal and premotor cortices are involved in adapting walking and running speed on the treadmill: an optical imaging study. *Neuroimage, 23*(3), 1020-1026.

Taga, G., Asakawa, K., Hirasawa, K., & Konishi, Y. (2003). Hemodynamic responses to visual stimulation in occipital and frontal cortex of newborn infants: a near-infrared optical topography study. *Early Human Development, 75*, S203-S210. doi: 10.1016/j.earlhumdev.2003.08.023

Telkemeyer, S., Rossi, S., Koch, S. P., Nierhaus, T., Steinbrink, J., Poeppel, D., . . . Wartenburger, I. (2009). Sensitivity of newborn auditory cortex to the temporal structure of sounds. *The Journal of Neuroscience, 29*(47), 14726-14733.

Thomas, R., & Stephane, P. (2008). Prefrontal cortex oxygenation and neuromuscular responses to exhaustive exercise. *European journal of applied physiology, 102*(2), 153-163.

Thompson, C., Wylie, L. J., Fulford, J., Kelly, J., Black, M. I., McDonagh, S. T., . . . Jones, A. M. (2015). Dietary nitrate improves sprint performance and cognitive function during prolonged intermittent exercise. *European journal of applied physiology*, 1-10.

Tian, F., Sharma, V., Kozel, F. A., & Liu, H. (2009). Functional near-infrared spectroscopy to investigate hemodynamic responses to deception in the prefrontal cortex. *Brain Research, 1303*, 120-130. doi: 10.1016/j.brainres.2009.09.085

Toda, N., Ayajiki, K., & Okamura, T. (2009). Cerebral Blood Flow Regulation by Nitric Oxide: Recent Advances. *Pharmacological Reviews, 61*(1), 62-97. doi: 10.1124/pr.108.000547

Tong, Y., Rooney, E. J., Bergethon, P. R., Martin, J. M., Sassaroli, A., Ehrenberg, B. L., . . . Fantini, S. (2005). Studying brain function with near-infrared spectroscopy

concurrently with electroencephalography. *Optical Tomography and Spectroscopy of Tissue VI, 5693*, 444-449. doi: 10.1117/12.587662

Toronov, V., Webb, A., & Choi, J. H. (2001). Investigation of human brain hemodynamics by simultaneous near-infrared spectroscopy and functional magnetic resonance imaging. *Medical Physics, 28*(4), 521-527.

Troubat, N., Fargeas-Gluck, M., Tulppo, M., & Dugue, B. (2009). The stress of chess players as a model to study the effectsof psychological stimuli on physiological responses: an example of substrate oxidation and heart rate variability in man. *European journal of applied physiology, 105*, 343-349.

Umemura, T., Ueda, K., Nishioka, K., Hidaka, T., Takemoto, H., Nakamura, S., . . . Higashi, Y. (2006). Effects of acute administration of caffeine on vascular function. *American Journal of Cardiology, 98*(11), 1538-1541. doi: 10.1016/j.amjcard.2006.06.058

Van Beek, T. A. (2002). Chemical analysis of Ginkgo biloba leaves and extracts. *Journal of Chromatography A, 967*(1), 21-55.

Vanhatalo, A., Bailey, S. J., Blackwell, J. R., DiMenna, F. J., Pavey, T. G., Wilkerson, D. P., . . . Jones, A. M. (2010). Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 299*(4), R1121-R1131. doi: 10.1152/ajpregu.00206.2010

Villringer, A., & Chance, B. (1997). Non-invasive optical spectroscopy and imaging of human brain function. *Trends in Neurosciences, 20*, 435-442.

Wallois, F., Mahmoudzadeh, M., Patil, A., & Grebe, R. (2012). Usefulness of simultaneous EEG-NIRS recording in language studies. *Brain and Language, 121*(2), 110-123. doi: 10.1016/j.bandl.2011.03.010

Watanabe, A., Kato, N., & Kato, T. (2002). Effects of creatine on mental fatigue and cerebral hemoglobin oxygenation. *Neuroscience Research, 42*(4), 279-285. doi: Pii s0168-0102(02)00007-x

Webb, A. J., Patel, N., Loukogeorgakis, S., Okorie, M., Aboud, Z., Misra, S., . . . Ahluwalia, A. (2008). Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension, 51*(3), 784-790. doi: 10.1161/hypertensionaha.107.103523

Weinmann, S., Roll, S., Schwarzbach, C., Vauth, C., & Willich, S. N. (2010). Effects of Ginkgo biloba in dementia: systematic review and meta-analysis. *BMC geriatrics, 10*(1), 14.

Wesnes, K. A. (2010). Evaluation of techniques to identify beneficial effects of nutrition and natural products on cognitive function. *Nutrition Reviews, 68*(11), S22-S28. doi: 10.1111/j.1753-4887.2010.00328.x

Wightman, E. L. (2013). *The cognitive and cerebral blood flow effects of the polyphenol resveratrol in healthy, young humans.* (Unpublished PhD thesis), Northumbria University, Newcastle upon Tyne.

Wightman, E. L., Haskell-Ramsay, C. F., Reay, J. L., Williamson, G., Dew, T., Zhang, W., & Kennedy, D. O. (2015). The effects of chronic trans-resveratrol supplementation on aspects of cognitive function, mood, sleep, health and cerebral blood flow in healthy, young humans. *British Journal of Nutrition, 114*(09), 1427-1437.

Wightman, E. L., Haskell-Ramsay, C. F., Thompson, K. G., Blackwell, J. R., Winyard, P. G., Forster, J., . . . Kennedy, D. O. (2015). Dietary nitrate modulates cerebral blood flow parameters and cognitive performance in humans: A double-blind, placebo-controlled, crossover investigation. *Physiology & behavior, 149*, 149-158.

Wightman, E. L., Haskell, C. F., Forster, J. S., Veasey, R. C., & Kennedy, D. O. (2012). Epigallocatechin gallate, cerebral blood flow parameters, cognitive performance and mood in healthy humans: a double-blind, placebo-controlled, crossover investigation. *Human Psychopharmacology: Clinical and Experimental, 27*(2), 177-186.

Wightman, E. L., Reay, J. L., Haskell, C. F., Williamson, G., Dew, T. P., & Kennedy, D. O. (2014). Effects of resveratrol alone or in combination with piperine on cerebral blood flow parameters and cognitive performance in human subjects: a

randomised, double-blind, placebo-controlled, cross-over investigation. *British Journal of Nutrition, 112*(02), 203-213.

Winther, K., Randlov, C., Rein, E., & Mehlsen, J. (1998). Effects of Ginkgo biloba extract on cognitive function and blood pressure in elderly subjects. *Current Therapeutic Research-Clinical and Experimental, 59*(12), 881-888. doi: 10.1016/s0011-393x(98)85053-4

Wu, Y., Li, S., Cui, W., Zu, X., Du, J., & Wang, F. (2008). Ginkgo biloba extract improves coronary blood flow in healthy elderly adults: Role of endothelium-dependent vasodilation. *Phytomedicine, 15*(3), 164-169. doi: 10.1016/j.phymed.2007.12.002

Wylie, L. J., Kelly, J., Bailey, S. J., Blackwell, J. R., Skiba, P. F., Winyard, P. G., . . . Jones, A. M. (2013). Beetroot juice and exercise: pharmacodynamic and dose-response relationships. *Journal of Applied Physiology, 115*(3), 325-336.

Yamaguchi, T., Kanno, I., Uemura, K., Shishido, F., Inugami, A., Ogawa, T., . . . Suzuki, K. (1986). Reduction in regional cerebral metabolic rate of oxygen during human aging. *Stroke, 17*(6), 1220-1228.

Yang, M., Xu, D. D., Zhang, Y., Liu, X., Hoeven, R., & Cho, W. C. S. (2014). A Systematic Review on Natural Medicines for the Prevention and Treatment of Alzheimer's Disease with Meta-Analyses of Intervention Effect of Ginkgo. *The American Journal of Chinese Medicine, 42*(03), 505-521.

Yeo, S. E., Jentjens, R., Wallis, G. A., & Jeukendrup, A. E. (2005). Caffeine increases, exogenous carbohydrate oxidation during exercise. *Journal of Applied Physiology, 99*(3), 844-850. doi: 10.1152/japplphysiol.00170.2005

Yokogoshi, H., Kato, Y., Sagesaka, Y. M., Takiharamatsuura, T., Kakuda, T., & Takeuchi, N. (1995). Reduction Effect Of Theanine On Blood-Pressure And Brain 5-Hydroxyindoles In Spontaneously Hypertensive Rats. *Bioscience Biotechnology and Biochemistry, 59*(4), 615-618.

Yokogoshi, H., Kobayashi, M., Mochizuki, M., & Terashima, T. (1998). Effect of theanine, r-glutamylethylamide, on brain monoamines and striatal dopamine release in conscious rats. *Neurochemical Research, 23*(5), 667-673.

Yoto, A., Motoki, M., Murao, S., & Yokogoshi, H. (2012). Effects of L-theanine or caffeine intake on changes in blood pressure under physical and psychological stresses. *J Physiol Anthropol, 31*, 28.

Young, G. B. (2000). The EEG in coma. *Journal of Clinical Neurophysiology, 17*(5), 473-485.

Yun, T. K. (2001). Panax ginseng--a non-organ-specific cancer preventive? *The lancet oncology, 2*(1), 49-55. doi: 10.1016/s1470-2045(00)00196-0

Appendices

Appendix A

PARTICIPANT INFORMATION.

TITLE OF PROJECT: __ Investigation of the acute effects of two doses of *Ginkgo biloba* on cerebral blood flow cognitive performance and mood.

Participant ID Number:

Principal Investigator: __Fiona Dodd_____

Investigator contact details: Email: fiona.dodd@unn.ac.uk

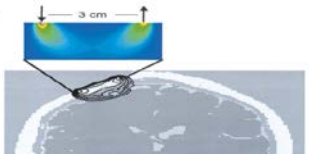
This project is funded by: BPNRC

Number of participant points / payment: £72

INFORMATION TO POTENTIAL PARTICIPANTS

1. What is the purpose of the project?

Ginkgo biloba is a common dietary supplement that has been taken historically to enhance alertness, is recognised as safe and can be obtained from most health food shops. The aim of the proposed study is to assess the effects of single administration of two different doses of *Ginkgo biloba* on cerebral blood flow, cognitive performance and mood, in comparison to placebo. Cerebral blood flow will be measured using Near Infrared Spectroscopy (NIRS). NIRS involves wearing a non-invasive headband across the forehead that uses light emission and absorption to measure blood flow (see diagram below). Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.



2. Why have I been selected to take part?

You have been selected because you are a healthy adult aged 18-40 years. You have indicated that you are healthy, do not habitually smoke (more than 3 cigarettes a day), are not currently taking any prescription or over-the-counter medicines (excluding the contraceptive pill) or illicit social drugs and you are not currently taking any dietary or herbal supplements. You are proficient in English equivalent to a native English speaker.

3. What will I have to do?

You will need to attend the laboratory on four separate days, each separated by a 1 week period. The first time will be for screening/training, the following three visits will be treatment visits. On each of these treatment visits you will attend the laboratory around midday, and remain in the lab for approximately 3.5hours (see below).

Screening/Training Visit: At the screening visit you will be screened by the researcher to confirm your eligibility to take part in the study and undergo training on the computer tasks. The format of the session will be the same as for the testing sessions. The tasks will last for approximately 30 minutes and will assess attention and performance; they do not measure any aspect of intelligence or personality. You will complete the tasks 3 times in order to familiarise yourself with them. You will also be asked to complete some mood scales. Demographic data such as height and weight will also be taken. Before leaving you will be provided with a food diary. This visit will take approximately 1.5 hours.

Study visit 1: This will take place after the initial screening/training visit. Upon arrival you will meet with the researcher and confirm your compliance to any study restrictions and hand in your completed food diary which will document what you have had for breakfast that morning. If you usually have a cup of tea or coffee in the morning you must consume this 1.5 hours before testing. You should have your breakfast and coffee or tea (if you normally consume it) 1 hour and 30 minutes prior to your study visit. You will then have your blood pressure and heart rate taken and complete a set of mood scales and following this will be provided with a light snack (a choice of cheese or ham sandwich). The NIRS headband will then be fitted across your forehead in order for brain activity to be monitored (please see section 1 for further information regarding how the NIRS is used to measure your brain activity). You will then begin the cognitive tasks, which will provide your baseline performance for the day (identical to those completed on your training day and will take approximately 20 minutes). Once you have completed the tasks the NIRS will begin recording and you will have a 10 minute rest period where you will be required to sit quietly. Following this you will be given your treatment to take for the day. You will then remain in the testing laboratory with the headband on for a further 90 minutes whilst readings are taken at rest (you will watch a DVD during this time). Following this period you will complete six repetitions of the same battery of tasks. You will then have your blood pressure and heart rate taken and complete a set of mood scales for the second time. The procedures will be conducted by appropriately trained staff. All procedures have been risk-assessed. Should the tests reveal an abnormality (where recognised clinical guidelines regarding test results exist) the researcher will recommend to you that you seek further medical advice from your GP, bear in mind though that a single test may not always provide an accurate reflection of your health status. The whole visit will last approximately 3.5 hours.

Study visits 2 and 3 will be exactly the same as visit 1 apart from the treatment that is received.

- You will need to turn up at the laboratory promptly on the agreed days and at the agreed times.
- You will need to abstain from alcohol from 8pm the evening before your treatment visits until you have completed your visit to the lab.
- If you occasionally smoke you will also need to abstain from smoking on the morning of your visits until the end of the testing day.
- You will need to perform the computer tasks to the best of your abilities.

- You will need to consider your response to each item on the mood scales carefully.
- You will not undertake any exercise on the day of the study visits until the visit is complete.
- You will need to agree not to take any herbal or dietary supplements whilst you are participating in the study. If prior to, or during, your participation in this study you are prescribed medication you need to notify the research team as soon as possible as it may impact upon your ability to take part in the study.
- You will also need to maintain a similar lifestyle and nutrition to what you did prior to the study.

4. What are the exclusion criteria (i.e. are there any reasons why I should not take part)?

You should not take part if you are allergic to *Ginkgo biloba*. You are not eligible to take part in this study if you: are not proficient in English, are (or are seeking to become) pregnant, are currently taking illicit, or over the counter/prescription medication (excluding the contraceptive pill), and/or dietary/herbal supplements. You are also ineligible if you have any food allergies or sensitivities that are relevant to the study and if you have a history of/current head trauma, learning difficulties, ADHD and migraines, gastric problems, dyslexia or colour blindness. This will be discussed/confirmed at the initial screening session along with other relevant health issues that may impact upon your eligibility for the study. If you are unsure about your eligibility then please email me and we can discuss any issues or ambiguity

5. Will my participation involve any physical discomfort?

You will be required to wear a headband across the forehead that holds the NIRS optodes in place. Because you have to keep the headband on for a period of 3.5 hours it may cause minor discomfort. The study will also involve long periods of sitting in front of a computer screen which you may find uncomfortable. These procedures have been assessed for potential risks and deemed to be minor.

6. Will my participation involve any psychological discomfort or embarrassment?

No

7. Will I have to provide any bodily samples (i.e. blood, saliva)?

No

8. How will confidentiality be assured?

The research team has put into place a number of procedures to protect the confidentiality of participants. These include:

Allocation of an individual participant code that will always be used to identify any data provided. Your name or other personal details will not be associated with your data, for example the consent form that you sign will be kept separate from your performance data.

All paper records will be stored in a locked filing cabinet, accessible only to the research team, and all electronic information will be stored on a password-protected computer. All of the information you provide will be treated in accordance with the Data Protection Act.

9. Who will have access to the information that I provide?

The principal investigator and members of the research team will have access to the information that you provide. However, any data that leaves the site will only be identifiable by a study number and it will not be possible for anybody outside of the investigational site to connect your identifying information to your data.

10. How will my information be stored / used in the future?

It is intended that the results of the study will eventually be published in a peer-reviewed journal. In the meantime, if you wish to find out what effect the treatments had, we will email a summary of the results to you approximately two weeks after the study finishes. At no time will you personally be identified as having taken part. We will not be able to provide any information on your own individual performance. All information and data gathered during this research will be stored in line with the Data Protection Act and will be destroyed 5 years following the conclusion of the study.

11. Has this investigation received appropriate ethical clearance?

Yes, this study has received ethical approval from the School of Psychology and Sports Science Ethics committee. If you require confirmation of this please contact the Chair of this Committee, stating the title of the research project and the name of the principle investigator:

Chair of School of Psychology & Sport Science Ethics Committee, Northumberland Building, Northumbria University, Newcastle upon Tyne, NE1 8ST

12. Will I receive any financial rewards / travel expenses for taking part?

You will receive £72 for taking part in the study to cover your out of pocket expenses and potential loss of earnings. If you decide to drop out of the study you will still be paid for the days that you have completed (at the discretion of the researcher). **We will endeavour to have your payment available for you on the day you complete the study, however, this may not always be possible.** If your payment is not available on your final study day then the researcher will let you know when you can expect to receive it.

13. How can I withdraw from the project?

You are free to withdraw from the study for any reason and do not have to disclose this reason to the investigators. If you wish to withdraw your data please contact the investigator within a month of your participation. After this date, it may not be possible to withdraw your individual data as the results may already have been published. However, as all data are anonymised, your individual data will not be identifiable in any way.

14. If I require further information who should I contact and how?

If you need more information, would like to discuss your participation, or experience any problems as a consequence of taking part in the study you should contact: Fiona Dodd (fiona.dodd@unn.ac.uk) in the Brain, Performance and Nutrition Research Centre.

Appendix B

General Health Screen

Please read the following list carefully. You are not eligible to participate in the research if:

1. You have history of neurological, vascular or psychiatric illness (excluding depressive illness and anxiety).
2. You have learning difficulties, dyslexia or colour blindness.
3. You have visual impairment that cannot be corrected with glasses or contact lenses.
4. You have a current diagnosis of depression and/or anxiety.
5. You have a history or current diagnosis of drug/alcohol abuse.
6. You have migraines.
7. You have anaemia.
8. You have a heart disorder.
9. You have high blood pressure.
10. You have respiratory disorder.
11. You have type-I diabetes.
12. You have food intolerances/sensitivities.
13. You have phenylketonuria.
14. You are pregnant or seeking to become pregnant.
15. You smoke more than 3 cigarettes per day.
16. You are currently taking any prescribed (excluding contraceptives), illicit or herbal drugs.

If you are unsure or wish to discuss any of these points with the researcher then you are welcome to do so.

Appendix C

Caffeine Consumption

Subject No._____ Subject Initials_____

Do you drink coffee?……Yes ☐ **No** ☐

How often do you drink CAFFEINATED coffee? (Normal coffee)

	Tick one box
Every day	
Most days	
Some days	
Hardly ever	
Never	

If you drink coffee regularly (Every Day or Most Days)

Quantity per day……

Or if you only drink coffee occasionally (Some Days or Hardly Ever)

Quantity per week…….

How often do you drink DECAFFEINATED coffee?

	Tick one box
Every day	
Most days	
Some days	
Hardly ever	
Never	

If you drink decaf coffee regularly (Every Day or Most Days)

Quantity per day…….

Or if you only drink decaf coffee occasionally (Some Days or Hardly Ever)

Quantity per week…….

Do you drink tea?……Yes ☐ **No** ☐

How often do you drink CAFFEINATED tea? (Normal tea)

	Tick one box
Every day	
Most days	
Some days	
Hardly ever	
Never	

If you drink tea regularly (Every Day or Most Days)

Quantity per day……..

Or if you only drink tea occasionally (Some Days or Hardly Ever)

Quantity per week……

How often do you drink DECAFFEINATED tea? (don't include Herbal Tea)

	Tick one box
Every day	
Most days	
Some days	
Hardly ever	
Never	

If you drink decaf tea regularly (Every Day or Most Days)

Quantity per day……

Or if you only drink decaf tea occasionally (Some Days or Hardly Ever)

Quantity per week…….

Do you drink soft drinks?……Yes ☐ **No** ☐

How often do you drink CAFFEINATED soft drinks? (e.g. coke, pepsi, lucozade)

	Tick one box
Every day	
Most days	
Some days	
Hardly ever	
Never	

If you drink caff. soft drinks regularly (Every Day or Most Days)

Quantity per day…….

Or if you only drink caff. soft drinks occasionally (Some Days or Hardly Ever)

Quantity per week……

How often do you drink NON-CAFFEINATED soft drinks? (e.g. fruit juice, squash, lemonade, orangeade etc)

	Tick one box
Every day	
Most days	
Some days	
Hardly ever	
Never	

If you drink non-caff. soft drinks regularly (Every Day or Most Days)

Quantity per day……..

Or if you only drink non-caff. soft drinks occasionally (Some Days or Hardly Ever)

Quantity per week…….

If you never drink caffeinated drinks is there any particular reason for this?…… Yes ☐ No ☐

If yes, please give details

_____
_____
_____
_____